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PROCEEDINGS

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THE PROCEEDINGS OF THE ZOOLOGICAL SOCIETY OF LONDON

THE TRANSACTIONS OF THE ZOOLOGICAL SOCIETY OF LONDON

Editor . . . . . L. HARRISON MATTHEWS, F.R.S.

Clerk of Publications . . . G. B. STRATTON, M.B.E.

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REPORT OF THE PATHOLOGIST FOR THE YEAR 1960

BY

R. N. T.-W.-FIENNES, M.A., M.R.C.V.S.

Zoological Society of London

[Accepted 13th June, 1961]

An account is given of autopsy examinations on Mammalia, Aves and Reptilia during the year 1960. Statistical analyses of results are added in the tables given. Various research problems studied during the year are briefly described.

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GENERAL

Reference has been made in previous reports to the introduction of new recording systems. During the year under review the new systems have been re-examined with the assistance of Dr F. Yates, Director of Statistics at Rothamsted. As a result, and in view of new plans to provide the Nuffield Institute of Comparative Medicine, it was decided that additional clerical assistance should be afforded for these laboratories with the following objects :—

1. To transfer the Society's past pathological records to the newly-introduced punch card system thus rendering them capable of analysis over a period of some fifty years.
2. To compile an index of world literature of comparative medicine.
3. In due course to prepare the necessary statistical analyses for proper co-ordinated study.

In addition two index collections are being prepared for purposes of comparative study. These are :—

1. A collection of colour transparencies illustrating pathological conditions found in the various groups of animals.
2. A collection of histological material stored in paraffin blocks from normal and abnormal organs of different groups of animals of different sexes and at different ages where known.

It is intended that this work shall be well advanced by the time the new Nuffield Institute of Comparative Medicine is inaugurated.

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It is intended that this work shall be well advanced by the time the new Nuffield Institute of Comparative Medicine is inaugurated.

During the year there have been no outbreaks of epidemic disease at Regent's Park. Moreover, diseases which regularly cause losses have been much diminished. Such are :— Enteritis of infectious origin amongst both birds and mammals, tuberculosis amongst Primates and Rodents, mycosis amongst birds and amoebiasis and nematodiasis amongst snakes.

It is evident that the health surveys made on groups of animals and described in previous reports have been successful in eliminating " carrier " animals and thus in diminishing the incidence of infection. Furthermore, there has been a satisfactory reduction of the death rate in newly-imported animals due to an extension of the diagnostic procedures to detect " carrier " animals and to the use of tranquillisers during the acclimatization period.

The comparative results for the year are recorded in Tables 1 to 6 (pp. 187-196). Cases of particular interest are recorded in the next section.

CASE HISTORIES

The following is an account of the more interesting cases investigated *post mortem*.

MAMMALIA

Primates

DB R54/60. Indonesian Gibbon (*Hylobates moloch*).
Sex female. Age unknown. Length of stay at Regent's Park five months.

The Indonesian gibbon died as a result of chronic colitis caused by infection with *Entamoeba histolytica*.

DB 372/60 and DB 335/60. Indonesian Gibbon (*Hylobates moloch*).
Sex female. Age unknown. Length of stay at Regent's Park twelve months and thirteen months.

The two gibbons, received during September and October 1959, died within three days of each other as a result of enteritis. Both were found to be infected with *Shigella flexneri 103*. Death was, therefore, recorded as being due to enteritis caused by *Shigella* infection.

DB 48/60. Mandrill (*Mandrillus sphinx*).
Sex female. Age unknown. Duration of stay at Regent's Park nine years and two months.

The mandrill died twelve hours after operation for removal of a full-term foetus. The baby was removed alive, but died twenty-four hours after the death of the mother. The mother had given birth successfully to two babies in preceding years, but on this occasion suffered from dystokia. At *post mortem* she was found to be seriously affected with tuberculosis. One lobe of the lungs was solid and contained numerous fibrous nodes throughout its substance. The spleen was studded with pea-sized tubercules. Cultures were made from the spleen and *Mycobacterium tuberculosis* of human type was isolated.

DB 123/60. Squirrel Monkey (*Saimiri sciurea*).
Sex male. Age unknown. Duration of stay at Regent's Park ten months.

The squirrel monkey suffered from a rectal prolapse secondary to haemorrhagic enteritis. On culture an organism of *Shigella* sp. was isolated. The

organism could not be further identified because of contamination of cultures with *Proteus*.

DB R174/60. Brown Capuchin Monkey (*Cebus fatuellus*).
Sex male. Age approximately four years.

The monkey was received for *post mortem* from external sources. It died as a result of acute glomerulo-nephritis accompanied by large quantities of transudate in abdomen, thorax and pericardium. Cultures from kidney and liver revealed the presence of *Salmonella* sub-group B. Diagnosis acute nephritis resulting from salmonellosis.

DB R190/60. Brown Capuchin (*Cebus fatuellus*).
Sex male. Immature. Received for *post mortem* from external sources.

The capuchin died as a result of haemorrhagic enteritis associated with *Salmonella typhimurium* infection.

DB 54/60. Common Marmoset (*Hapale jacchus*).
Sex male. Age unknown. Duration of stay at Regent's Park thirteen months.

The marmoset suffered from fracture of the tail complicated by a condition of shock. The adrenal glands were enlarged, friable and both contained haemorrhages.

Common Marmoset (*Hapale jacchus*).
Sex male. Age unknown. Received for *post mortem* from external sources.

The marmoset suffered from enteritis, there being numerous thickened zones throughout the length of the large intestine. Microscopical examination revealed these to be caused by encapsulated nematodes of very small size. Diagnosis—nodular worm disease.

DB 13/60. Cotton-headed Tamarin (*Oedipomidas oedipus*).
Sex female. Age unknown. Duration of stay at Regent's Park ten months.

The death of this tamarin was due to intestinal obstruction resulting from the presence of two thorny-headed worms in the gut (*Acanthocephala*). One of these was found attached to the wall of the ileum. The second worm had its head buried in the ileo-caecal valve. There was much thickening of the mucous membrane at the point of attachment of the worms, and this caused complete blockage of the intestine.

DB 293/60. Brown Lemur (*Lemur fulvus*).
Sex female. Age unknown. Duration of stay at Regent's Park six days.

The brown lemur had recently been received and was in a condition of near full-time pregnancy. She was grossly obese, and *post mortem* indicated that she was in a state of severe shock at the time of death. There was gross hyperaemia of all abdominal organs. The liver showed the typical congestive condition seen in shock cases. There were several small haemorrhages in the right adrenal. The lungs also showed the typical collapsed and haemorrhagic condition associated with shock conditions.

DB R142/60. Bush Baby (*Galago* sp.).
Sex not recorded. Age adult. Received for *post mortem* from external sources.

The *Galago* suffered from a dislocation of the tail vertebrae. Death was due to rupture of the liver and internal haemorrhage. Bacterial cultures from the

guts revealed the presence of *Shigella schmitzii*. Death was recorded as being due to injury associated with rupture of the liver. There was nothing to show that the *Shigella* infection was associated with pathological symptoms.

DB 69/60. Thick-tailed Bush Baby (*Galago crassicaudatus lasiotis*).
Sex male. Age unknown. Duration of stay at Regent's Park nine years and two months.

The *Galago* suffered from excessive obesity. The liver was large and friable, mottled brown and bright yellow in colour. Sections showed severe fatty infiltration. Death was recorded as being due to terminal hepatic failure.

DB 74/60. Bornean Slow Loris (*Nycticebus coucang*).
Sex male. Age adult. Duration of stay at Regent's Park four years and nine months.

The slow loris was much emaciated. The caecum was inflamed and the mucous membrane was affected with numerous rather deep ulcers. No other abnormalities were found, and death was attributed to inanition resulting from this ulceration. Cultures did not reveal the cause of the condition, only paracolon bacilli and yeast-like organisms being isolated.

Insectivora

DB 358/60. Moon Rat (*Echinosorex gymnura*).
Sex female. Adult. Duration of stay at Regent's Park four-and-a-half years.

Death was due to obstruction of the alimentary tract by an accumulation of hairs felted together in a mass and forming a complete cast of the stomach cavity. Diagnosis—death due to impaction of the stomach as a result of fur chewing.

DB 397/60. European Hedgehog (*Erinaceus europaeus*).
Sex male. Duration of stay at Regent's Park sixteen months.

The hedgehog was very fat and in good condition. It was evidently at the beginning of hibernation and all regions of the body contained abundant very soft fat. There was a large hibernation gland present in the scapular region ; the thymus was also very large. There was a large neoplasm of adenomatous type on the apex of the left adrenal gland together with metastases in the liver.

Carnivora

DB 351/60. Lion (*Panthera leo*).
Sex male. Immature. Duration of stay at Regent's Park ten days.

The lion cub died as a result of feline enteritis.

DB 316/60. Lion (*Panthera leo*).
Sex female. Adult. Duration of stay at Regent's Park seven years and ten months.

The lioness "Anne" was destroyed painlessly because of muscular dystrophy and weakness of the hind limbs. This was one of the lions which had been the subject of research by Fiennes and Jones (1960) in connection with osteodystrophy arising from calcium phosphorus imbalance. She had at one time been affected with the classical symptoms of this disease in an advanced form. There had been gross weakness of the hind limbs, inco-ordination and stargazing. As a result of treatment she had sufficiently improved to be

returned on exhibition, but some weakness of the back and hind limbs had always been detectable. Symptoms had returned in a more aggravated form rendering her retention impossible. At *post mortem* there were no signs of active disease though deformities of the skeleton were present which indicated its previous existence. Many of the bones indeed were abnormally dense and it appeared as if when calcium was available to the animal it had stored excess in the skeleton. A residual deformity was discovered in the skull since the foramen magnum was much narrowed and had exerted pressure on the spinal cord at the point of exit from the skull. It was evident that the final paralytic symptoms were due to this cause.

DB 128/60. Lion (*Panthera leo*)
Sex male. Adult. Duration of stay at Regent's Park four years and nine months.

The estimated age of this lion " Rusty " was five to six years. He died as a result of generalized tuberculosis affecting both lungs and both kidneys. The stomach and small intestine showed massive infestation with nematode worms (*Belascaris*).

DB 70/60. Lion (*Panthera leo*).
Sex female. Adult. Duration of stay at Regent's Park eight years and two months.

This lion " Amber " died as a result of tuberculosis of the small intestine. There were three bleeding ulcers about two inches apart in the organ one of which had almost perforated. Acid fast bacilli were cultured from the ulcers.

DB 45/60. Lion (*Panthera leo*).
Sex female. Adult. Duration of stay at Regent's Park nearly twenty-two years.

The lioness " Janet " was destroyed painlessly because of senility. She was found to be suffering from abdominal tuberculosis and there were two to three gallons of ascitic fluid in the abdominal cavity. The peritoneum was closely covered with pearl lesions of tuberculosis. Both lungs were small, collapsed and almost black with anthracotic desposits.

DB 362/60. Jungle Cat (*Felis chaus*).
Sex female. Adult. Duration of stay in Regent's Park one month.

The jungle cat died as a result of pneumonia affecting the right lung ; this was associated with infection by *Pseudomonas aeruginosa*.

DB 213/60. Jungle Cat (*Felis chaus*).
Sex female. Young adult. Duration of stay in Regent's Park three months.

The jungle cat was painlessly destroyed on account of injury to the right hind leg. All internal organs were normal, but the animal was found to be suffering from typical rickets of the type classically associated with Vitamin D deficiency.

DB 323/60. Small Indian Civet (*Viverricula indica*).
Sex male. Adult. Duration of stay in Regent's Park six months.

The civet was found to be suffering from diaphragmatic hernia, the stomach and small intestine being invaginated into the thoracic cavity by way of the oesophageal hiatus.

DB 192/60. Small Indian Mongoose (*Herpestes auropunctatus*).
Sex male. Adult. Duration of stay in Gardens eight years and two months.

The mongoose died as a result of septicaemia associated with infection by haemolytic streptococci. The entire length of the gut was full of food material and quantities of unaltered blood, though no obvious bleeding points were found. The bladder was nearly full of urine which was blood stained. There was an extensive diffuse area of haemorrhage from the wall of the bladder extending into the neck of the urethra. There was also urethritis. The prostate of this animal was much enlarged.

DB 63/60. Tibetan Wolf (*Canis lupus chanco*).
Sex female. Adult. Duration of stay at Regent's Park five years and eight months.

The wolf died as a result of tuberculosis of liver and lungs. There were numerous pea-sized tubercles in one lobe of the liver. All lobes of the lungs were a mottled grey and dark red and contained numerous cavities filled with pus. The mesenteric glands were enlarged and purulent.

DB 336/60. Indian Otter (*Lutra perspicillata*).
Sex female. Adult. Duration of stay in the Gardens two years and one month.

Numerous small white tubercles were discovered in the liver and lungs of this animal, believed at first to be due to tuberculosis. Cultures, however, revealed the presence of *Staphylococcus albus* together with a fungus of *Penicillium* species. Pseudotuberculosis associated with *Penicillium* infection has been described (vide C. W. Dodge " Medical Mycology " pp. 639 and 641, Henry Kimpton, London, 1936) and the present case is supposedly one of this nature.

DB 356/60. Stoat (*Mustela erminea*).
Sex not recorded. Adult. Duration of stay in Gardens ten months.

This stoat died as a result of enteritis, *Pseudomonas aeruginosa* being isolated from the gut.

DB R154/60. Polecat (*Mustela putorius*).
Sex male. Adult. Received from external sources.

The polecat died as a result of abdominal tuberculosis. All mesenteric glands were much enlarged and acid fast bacteria were cultured from them. Examination of the skeleton showed that this animal was suffering from typical cystic osteodystrophy of the " renal rickets " type. There had possibly been tuberculous infection of the kidneys and the disease may have been of the type described by Fiennes (1960) in a puma.

DB 221/60. Polecat-Ferret (*Mustela putorius*).
Sex female. Adult. Duration of stay in Regent's Park ten months.

The polecat-ferret died as a result of pneumonia associated with infection of pneumococcus and corynebacterium from lungs and blood.

DB 298/60. Yellow-throated Marten (*Martes flavigula*).
Sex female. Adult. Duration of stay in Regent's Park ten years.

There were numerous healed wounds on the head and neck of this animal which had been inflicted by its companions. The presence of numerous petechial haemorrhages in the cortex of both adrenal glands indicated a con-

dition of shock. In addition the kidneys showed interstitial nephritis and there were small pneumonic patches at the base of both lungs. It would appear that this animal, in poor health as a result of chronic nephritis, had been attacked by its companions, and that following injury, a state of shock had supervened associated with terminal pneumonia.

DB 79/60. Libyan Striped Weasel (*Poecilictis libyca*).
Sex female. Adult. Duration of stay in Gardens sixteen months.

The weasel died as a result of enteritis affecting particularly the small intestine. Cultures revealed the presence of *Salmonella typhimurium*.

DB 116/60. Black-footed Cacomistle (*Bassariscus sumichrasti*).
Sex male. Adult. Duration of stay in Gardens five months.

The cacomistle died as a result of pulmonary tuberculosis. Both lungs were completely solid with the colour and texture of white cheese. Acid fast bacilli were isolated in culture from lung tissue.

DB 26/60. Red Panda (*Ailurus fulgens*).
Sex female. Adult. Received from Whipsnade.

The red panda died as a result of pulmonary tuberculosis. Both lungs revealed the presence of widespread confluent caseous lesions.

DB 60/60 and DB 106/60. Red Pandas (*Ailurus fulgens*).
Sex female and male. Duration of stay at Regent's Park fifteen days and two months.

Both pandas died as a result of hard-pad infection, virus being isolated at Messrs Burroughs Wellcome Laboratories at Beckenham. Bacterial cultures revealed the presence of haemolytic streptococci in both animals and of pneumobacillus in 60/60.

Rodentia

DB 198/60. Canadian Beaver (*Castor canadensis*).
Sex male. Adult. Duration of stay in Gardens four years and eight months.

The Canadian beaver died as a result of pneumonia, the right lung being completely solid and the left lung congested. Cultures from the lung revealed the presence of pneumococcus.

DB 337/60. Golden Hamster (*Mesocricetus auratus*).
Sex female. Adult. Duration of stay in Gardens one month.

The hamster died as a result of ulceration of the stomach and intestines. There was a large ulcer of the stomach and several small ulcers in the duodenum.

DB 376/60. Coypu (*Myocastor coypus*).
Sex female. Adult. Duration of stay in Gardens four years and seven months.

The coypu suffered from a neoplasm of the mammary gland with metastasis of the right adrenal. The neoplasm was adeno-carcinomatous.

DB 112/60. Coypu (*Myocastor coypus*).
Sex male. Adult. Duration of stay in Gardens three years and eleven months.

The lungs contained numerous calcified nodes of varying size, the largest being the size of a pea. The chest cavity was filled with clear fluid. The pericardium was covered with nodes similar to those found in the lungs also calcified. A few nodes were also found in the myocardium. These nodes

proved to be neoplastic and of the nature of an osteosarcoma. No primary tumour was found in the skeleton.

DB 7/60. Coypu (*Myocastor coypus*).
Sex female. Adult. Duration of stay in Gardens three years and eight months.

The coypu was painlessly destroyed on account of a large mammary tumour. This proved to be adeno-carcinomatous, and there were metastases in liver, kidneys and adrenals.

DB 386/60. Spotted Paca (*Cuniculus paca*).
Sex male. Adult. Duration of stay in Gardens seventeen months.

The paca died as a result of enteritis associated with infection by *Escherichia coli* O. B. group B 12.

DB 68/60 and DB R353/60. European Hares (*Lepus europaeus*).
Both male. Adults. Duration of stay in Garden seven days and six months.

Both these hares died as a result of coccidial infection.

Perissodactyla

DB R68/60. Przewalski's Wild Horse (*Equus przewalskii*).
Sex male. Adult. Duration of stay at Whipsnade four years.

This horse died as a result of dislocation of the atlas vertebra. Examination of the heart revealed the presence of large vegetations on the muscular tissue beneath the aortic valves. The aorta showed numerous small gravel-like irregularities, possibly of medial origin.

DB R11/60. Grevy's Zebra (*Equus grevyi*).
Sex female. Subadult. Duration of stay at Whipsnade seven months.

The zebra died as a result of *Nuttalia equi* infection parasites being very numerous in the red blood corpuscles. Some zebras from the same consignment had been lost from this cause during the previous summer and treatment had been given to the remainder. There was a large cavernoma of parasitic origin affecting the anterior mesenteric artery.

DB R9/60. Brazilian Tapir (*Tapirus terrestris*).
Sex female. Adult. Received from Whipsnade.

The tapir died as a result of impaction of the caecum together with ulceration of the rectum. Cultures failed to reveal the presence of any pathogenic organisms in the gut contents.

Artiodactyla

DB 344/60. Cape Buffalo (*Syncerus caffer*).
Sex male. Aged about seven months. Duration of stay in Regent's Park one month.

The young buffalo died as a result of paratyphoid due to infection with *Salmonella* sub-group C.

DB 231/60. Beisa Oryx (*Oryx gazella beisa*).
Sex female. One-and-a-half months old. Born in the Menagerie.

The young oryx was infected with *Salmonella* sub-group E. There was also a concretion of curdled milk in the abomasum. This appears to have given rise to a condition of tympany and colic.

DB 110/60. Spotted Deer (*Axis axis*).
Sex female. Adult. Duration of stay in Regent's Park eight months.

The spotted deer died as a result of tuberculosis of lungs and gut. Both lungs were largely composed of caseous material with no apparent normal tissue left.

DB R271/60. Hippopotamus (*Hippopotamus amphibius*).
Sex female. One day old. Born at Whipsnade.

The hippopotamus calf died as a result of injuries sustained during parturition. It is interesting to note that the gut contents contained *Salmonella newport*.

Edentata.

DB 200/60. Seven-banded Armadillo (*Dasypus septemcinctus*).
Sex male. Adult. Duration of stay at Regent's Park eighteen months.

The armadillo died as a result of enteritis caused by infection of *Salmonella* 11 (Aberdeen).

Marsupialia.

DB 396/60. Black-faced Kangaroo (*Macropus canguru melanops*).
Sex male. Adult. Duration of stay in Regent's Park five months.

The kangaroo died of pneumonia associated with infection by streptococci.

DB 394/60. Kangaroo Island Kangaroo (*Macropus canguru fuliginosus*).
Sex female. Adult. Duration of stay at Regent's Park five months.

Death was due to nocardiosis with infection of both mandibles.

DB 61/60. Red Kangaroo (*Macropus rufus*).
Sex male. Adult. Duration of stay in Regent's Park twenty-five months.

This kangaroo was painlessly destroyed because of nocardiosis of the left mandible.

DB 287/60. Bennett's Wallaby (*Protemnodon rufogrisea frutica*).
Sex male. Adult. Duration of stay at Regent's Park five years and one month.

The wallaby died as a result of faecal impaction. There was medial sclerosis affecting the abdominal aorta.

DB 370/60. Dama Wallaby (*Protemnodon eugenii*).
Sex female. Adult. Duration of stay in Regent's Park five months.

Death was due to pneumonia affecting both lungs. There was a terminal intussusception.

DB 8/60. Squirrel-like Flying Phalanger (*Petaurus norfolcensis*).
Sex male. Adult. Duration of stay at Regent's Park was ten months.

The phalanger died as a result of gastritis and duodenitis associated with infection by *Pseudomonas aeruginosa*.

DB 183/60. Tasmanian Devil (*Sarcophilus harrisi*).
Sex male. Adult. Duration of stay in Gardens six years and two months.

The Tasmanian devil died as a result of septicaemia associated with streptococcal infection. The left adrenal gland was greatly enlarged and neoplastic. The animal also suffered from hepatitis.

AVES

DB 135/60. Great Indian Hornbill (*Buceros bicornis*).
Sex male. Adult. Duration of residence at Regent's Park nine years.

This hornbill was evidently an old bird. *Post mortem* showed that there were advanced lesions in the arterial system and kidneys. There was longitudinal streaking throughout the thoracic and abdominal aortae and in the brachio-cephalic trunk. The pulmonary artery was normal. In the abdomen the lesion took the form of a longitudinal ridge along the dorsal wall of the aorta stained lemon yellow. The kidneys were shrunken and sclerotic and indicated the presence of chronic interstitial nephritis.

DB 16/60. Piping Hornbill (*Bycanistes buccinator fistulator*).
Sex female. Adult. Duration of stay at Regent's Park eight-and-a-half years.

This was an aged bird and suffered from an advanced degree of atherosclerosis. All the main arteries at the point of exit from the heart for 2–3 cm. were inelastic and the calibre was reduced by atheromatous deposits.

DB 234/60 to 239/60 and 243/60. Kingfishers (*Alcedo atthis ispida*).
Fledglings. Duration of stay at Regent's Park about one month.

Seven kingfishers of different sexes had been brought as fledglings and all died within a few days of each other about one month later. In every case death was due to nephritis and cultures revealed the presence of *Salmonella* species in the kidneys.

DB 121/60. Budgerigar (*Melopsittacus undulatus*).
Sex male. Adult. Duration of stay at Regent's Park three years and seven months.

Amongst many budgerigars presented for *post mortem* the present case is worthy of note. The bird suffered from severe impaction of the hypopygeal (preen) gland to such an extent that it projected downwards into the body cavity resembling a tumour. This caused obstruction and impaction of the cloaca.

DB 255/60 to DB 257/60. King Penguins (*Aptenodytes patagonica*).
Two females and one male. Adult.

These king penguins together with a chick died after an exceptionally cold wet night during the month of August. The one male and one of the females were the parents of the chick and had been nursing it. The chick appeared to have died as a result of exposure following the death of the parents. All the penguins were in good condition and supplied with plenty of blubber. At *post mortem* all showed similar changes but in different degree. The livers were pale and streakily patched with superficial and deep splashed haemorrhages of ecchymotic type. The intestines were filled with liquid undigested blood. The carcases had a fevered appearance. Bacteriological culture revealed the presence of *Pasteurella multocida* and diagnosis was made of pasteurellosis. This organism was agglutinated to a titre of 1 : 10,000 by serum from the penguins.

DB 189/60. Black-footed Penguin (*Spheniscus demersus*).
Sex female. Adult. Duration of residence at Regent's Park four months.

This penguin, which was comparatively newly arrived in the Gardens, died

as a result of nephritis. The kidneys were pale with large amounts of retained urates. Cultures revealed the presence, both in guts and kidneys, of *Salmonella* sub-group C.

DB 277/60. Black-footed Penguin (*Spheniscus demersus*).
Sex female. Adult. Duration of stay in Regent's Park six months.

This penguin also had been in residence at Regent's Park only a short time. Death was due to mycosis which affected only the right air sac.

DB 78/60. Ceylonese Necklace Dove (*Streptopelia chinensis*).
Sex female. Adult. Duration of stay at Regent's Park three years and seven months.

This bird which had been resident in the Gardens for nearly four years eventually died as an extreme case of gout. Both legs and wings were greatly thickened as a result of uric acid deposits ranging along the synovia.

DB 248/60. Blue Bird of Paradise (*Paradisea rudolphi margaritae*).
Sex female. Adult. Duration of stay at Regent's Park two years and ten months.

The bird died as a result of congestion of the lungs and enteritis. Gut cultures yielded *Shigella newcastle*.

DB 266/60. Malayan Glossy Starling (*Aplonis panyensis strigatus*).
Sex male. Adult. Duration of residence at Regent's Park five years and seven months.

This bird died as a result of haemorrhagic enteritis. Cultures were made from the guts and yielded *Salmonella typhimurium* and *Shigella flexneri* X.

REPTILIA

DB 197/60. Greek Tortoise (*Testudo graeca*).
Sex female. Duration of stay at Regent's Park fifteen months.

This tortoise died, as is commonly the case, as a result of impaction of the large bowel with nematodes. A record is included because *Clostridium tetani* was isolated from a series of pinhead nodules present in the pharynx, oesophagus and cardia of the stomach.

DB 228/60. Reticulated Python (*Python reticulatus*).
Sex male. Duration of stay at Regent's Park ten months.

The python died as a result of enteritis. Intestinal cultures revealed the presence of *Salmonella* of sub-group D. *Salmonella* is usually non-pathogenic in reptiles, but they are often carriers of organisms of this group.

DB 227/60. Ribbon Snake (*Thamnophis sauritis*).
Sex female. Duration of stay at Regent's Park under two months.

This snake died as a result of dystokia due to the retention of twenty fully-developed foetuses. This specimen has been retained for demonstration purposes.

RESEARCH

ASPERGILLOSIS OF BIRDS

An analysis of deaths amongst birds during 1958–1960 shows that thirty-nine from nine hundred and eighty deaths resulted from mycosis. This gives a

figure of 4 per cent, but amongst penguins seven of twenty-four deaths were due to mycosis, making a total of 29 per cent. Mycosis is thus a serious source of loss amongst penguins, particularly amongst those that are newly imported and not yet acclimatised. The cost of these birds is high, and the loss is, therefore, serious. Table 6 gives an analysis of deaths from mycosis showing the species affected, the organs in which lesions were found and the type of lesion seen. Experience is showing that *Aspergillus* can often be isolated from abnormal sites by cultural methods, thus isolates have been obtained from the alimentary canal, the brain and from the beak of one penguin which showed hyperkeratosis. Isolates have also been made from livers, and in one case, from the myocardium in circumstances in which the lesion was diffuse and in which no plaques or nodes were seen. It is possible, therefore, that if more careful scrutiny of organs were made by cultural methods the incidence of infection might prove to be higher.

Two factors were considered to be of prime importance in the control of this infection :

1. The source of infection
2. The physical condition of the bird when imported.

A survey was, therefore, made of the Regent's Park Gardens in order to ascertain whether there existed dangerous foci of infection, and if so, what steps could be taken to control them. In this work generous assistance was afforded by Mr P. G. Austwick, Mycologist to the Veterinary Laboratory, New Haw, Weybridge and by Dr J. M. Hirst, Mycologist at Rothamsted, who loaned to the Society his Hirst spore counter for four to five months. Results with the spore counter revealed over this period a very low level of aerial contamination ; this may have been partly due to the unduly wet weather encountered during the summer. The only serious source of infection discovered was in hay bales in the cattle and antelope houses. In some cases grass had evidently been baled wet, and owing to fermentation at the centre of the bale, heavy contamination with *Aspergillus* was found. Hay bales must, therefore, be regarded as a potential source of infection unless these have been artificially dried within a week of baling. The same danger does not, of course, exist with hay obtained from a rick since fermentation from wet hay would then lead to conflagration.

On the evidence available it appeared most probable that penguins became infected with aspergillosis prior to arrival at Regent's Park. Once acclimatised they appear to be relatively resistant to infection, and are in any case not exposed to a high degree of risk. Control of the disease would, therefore, appear to depend on good treatment during the shipment period and to fortification of their resistance during the first months after arrival.

ENTERITIS IN BIRDS

It was suggested by Fiennes (1957) that one of the commonest causes of enteritis in birds was infection with *Escherichia coli*, an organism which was not a normal inhabitant of the intestines of birds which did not consume foods of animal origin including insects. Recently a start was made to type the

strains of *E. coli* involved in enteric cases amongst birds. Results obtained hitherto are as under :—

Psittaciformes. *E. coli communis* 7, *E. alkaligenes* 1, *E. coli* group O.B. 9, paracolon bacilli 1. Total 18.

Passeriformes. *E. coli communis* 4, *E. alkaligenes* (haemolytic) 2, *E. coli* group O.B. 4, paracolon bacilli 2. Total 12.

Thus various strains of the O.B. group of *E. coli* may be found associated with enteric disease in parrots and seed-eating birds, as also strains of *E. coli*, which in Mammalia would be regarded as non-pathogenic.

AMOEBIC INFECTION OF SNAKES

During the past three years Dr R. A. Neal of the Wellcome Laboratories of Tropical Medicine has been studying the pathogenesis and treatment of infections of *Entamoeba invadens* in snakes. The following information is published with his consent.

A comprehensive series of drugs was tested on *E. invadens* in vitro using culture material. Tests were made on cultures incubated for seven and fourteen days respectively. As a result of this work two drugs were selected for in vivo tests. These were Entamide and Fumagillin. Both these drugs were found effective in controlling and curing experimental *Entamoeba invadens* infections in grass snakes (*Natrix natrix* and *Thamnophis sirtalis*) at a dosage of 500 mg. per kilo. At autopsy the guts of treated snakes were found to be free from *Entamoeba* though cysts were found present in the liver of a snake treated with Entamide. No cysts were found in the liver of a snake treated with Fumagillin. Entamide was, however, selected as the drug of choice owing to its lesser toxicity, its readier availability and its cheapness.

Considerable difficulty has attended this work owing to the present scarcity of grass snakes, their availability only in the spring months, and the difficulty of keeping them alive under captive conditions. The work has thus been on a smaller scale than was hoped, but is not to be repeated unless Entamide proves unsatisfactory in routine practice.

CARDIO-VASCULAR DISEASES

Researches into the incidence of cardio-vascular diseases in wild animals and birds under captive conditions has been continued in collaboration with Dr R. Finlayson and Dr C. Symons. An analysis of the first seventy-five cases studied was presented at the Scientific Meeting of the Zoological Society of London in June 1960. An analysis of a further five hundred cases is now being prepared for publication.

STUDIES ON THE CHEMICAL COMPOSITION OF HAEMOGLOBINS FROM DIFFERENT GENERA

A comprehensive survey of haemoglobin chemistry in different genera is being made by Dr H. Lehmann by chromatographic and electrophoretic

methods. Results are now being studied and appear to have significance from a phylogenetic aspect.

ANATOMY OF THE POSTERIOR VENA CAVA IN DIFFERENT GENERA

Work on mechanisms of stress and shock, reported during the previous year, has been pursued by making comparative studies of the anatomy of the posterior vena cava in different genera of animals. In cases studied certain aspects of the stress syndrome have been difficult to understand ; it had appeared possible that in these cases some mechanism was concerned by which blood return to the heart was impeded by the action of the diaphragm on the posterior vena cava at the point of its passage into the thorax. Dissections were, therefore, made on different animals as material became available to determine the structure of the posterior vena cava and whether the relationship between this vessel and the diaphragm was such that the latter could, under certain conditions, impede the flow of blood to the heart.

The preliminary results of the investigation were presented at the Scientific Meeting of the Zoological Society during November 1960. It was there shown that, in all groups studied with the exception of Chiroptera the vena cava posterior to the diaphragm was somewhat dilated and in all cases wider than the portion found in the thorax. The relationships of the diaphragm to the vena cava varied considerably in different groups, but in all groups there was a firm tendinous attachment to the vena cava wall by which it was certain that during inspiration the calibre of the vessel must be altered and by which the dilated portion posterior to the diaphragm must be subjected to squeezing thus ensuring an onward movement of the blood. The relationship of the vena cava to the diaphragm was in contrast to that of the aorta and the oesophagus which pass through the diaphragm by way of a peritoneal investment ensuring that the diaphragm should slide along the tube whose position and calibre would be unaffected. It appeared evident, therefore, that in cases of shock in which the depth and rate of respiration was diminished the rate of blood return to the heart would be subjected to slowing with the consequent anaemia of the lung and congestion of the liver and other abdominal organs classically found in shock conditions.

A full account of this work is being prepared for publication.

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Table 1—Analysis of deaths occurring among acclimatised animals at Regent's Park during 1960.

	1959	1960					
		1st Qtr.	2nd Qtr.	3rd Qtr.	4th Qtr.	TOTAL	Nos. at 31.12.60
Mammals	147	34	24	26	36	120	725
Birds	254	45	71	53	52	221	1,539
Reptiles and Amphibia	93	12	14	13	12	51	581
TOTAL	494	91	109	92	100	392	2,845

Table 2—Analysis of the causes of death amongst the main classes of animals.

	Mammalia	Aves	Reptilia, etc.	TOTAL
Senility	1	8	0	9 (2%)
Inanition	0	1	8	9 (2%)
Injury, etc.	14	58	5	77 (20%)
Specific diseases	1	3	0	4 (1%)
General infections	7	6	5	18 (4%)
Tuberculosis	10	5	0	15 (4%)
Mycosis	3	1	0	4 (1%)
Parasitic	2	4	6	12 (3%)
Circulatory	3	6	4	13 (3%)
Alimentary	30	47	5	82 (21%)
Respiratory	18	23	4	45 (11%)
Urinary	4	41	8	53 (13·5%)
Reproductive	2	5	5	12 (3%)
Liver	5	1	1	7 (2%)
Nervous	1	0	0	1 —
Endocrine	0	0	0	0 —
Skin	0	1	0	1 —
Metabolism and nutrition	3	5	0	8 (2%)
Neoplasms	4	4	0	8 (2%)
Skeleton	2	0	0	2 (0·5%)
Other causes	10	2	0	12 (3%)
TOTAL	120	221	51	392

Table 3—Analysis of the causes of death amongst the main Orders of Mammalia.

	1	2	3	4	5	6	7	8	9	10	TOTAL
Senility	1	0	0	0	0	0	0	0	0	0	1
Inanition	0	0	0	0	0	0	0	0	0	0	0
Injury	3	0	2	0	4	0	7	0	0	0	16
Specific diseases	0	0	1	0	0	0	0	0	0	0	1
General infections	0	0	3	0	1	0	2	0	2	0	8
Tuberculosis	1	0	7	0	0	0	1	0	1	0	10
Mycosis	0	0	1	0	0	0	0	0	1	0	2
Parasitic	1	0	0	0	1	0	0	0	0	0	2
Circulatory	0	0	0	0	1	0	1	0	0	0	2
Alimentary	9	0	3	0	10	0	6	1	2	0	31
Respiratory	1	0	4	0	6	0	3	0	4	0	18
Urinary	0	0	3	0	1	0	0	0	0	0	4
Reproductive	0	0	0	0	1	0	1	0	0	0	2
Liver	1	0	3	0	1	0	0	0	0	0	5
Nervous	1	0	0	0	0	0	0	0	0	0	1
Endocrine	0	0	0	0	0	0	0	0	0	0	0
Skin	0	0	0	0	0	0	0	0	0	0	0
Metabolism and nutrition	1	0	1	0	1	0	0	0	0	0	3
Neoplasms	0	1	0	0	3	0	0	0	0	0	4
Skeleton	0	0	2	0	0	0	0	0	0	0	2
Other causes	1	1	0	1	2	0	0	0	3	0	8
TOTAL	20	2	30	1	32	0	21	1	13	0	120

1. Primates 20
2. Insectivora 2
3. Carnivora 30
4. Pinnipedia 1
5. Rodentia and Lagomorpha 32
6. Perissodactyla 0
7. Artiodactyla 21
8. Edentata 1
9. Marsupialia 13
10. Other Orders 0

TOTAL ..... 120.

Table 4—Showing identifications of infecting agents in *post mortem* specimens.

N.B. Inclusion in this list does not necessarily indicate that the organisms were the cause of death.

		Mammalia	Aves	Reptilia and Amphibia	TOTAL
BACTERIA TOTAL	Cocci	12	1	1	14
	Corynebacterium	2	1	1	4
	Acid Fast	6	4	0	10
	Pasteurella	2	3	0	5
	Salmonella	5	10	1	16
	Shigella	3	2	0	5
	Coliforms, etc.	5	14	2	21
	Brucella	0	0	0	0
	Anaerobes	1	0	1	2
	Pseudomonas	4	2	0	6
	Actinomyces	2	0	0	2
	Fungi	1	1	0	2
	Viruses	4	0	0	4
HELMINTHS TOTAL	Cestodes	0	1	1	2
	Nematodes	3	5	6	14
	Filaria	1	0	0	1
	Trematodes	0	0	0	0
PROTOZOA TOTAL	Sporozoa, etc.	0	0	1	1
	Trypanosomes	0	0	0	0
	Amoeba	0	0	1	1
	Coccidia	1	1	0	2
	Balantidium	0	0	0	0
	Other Protozoa	0	0	0	0
	Ecto-parasites	1	2	0	3
	TOTAL	53	47	15	115

Table 5—Analysis of causes of death amongst unacclimatised animals.

	Mammalia	Aves	Reptilia, etc.	TOTAL
Senility	0	0	0	0
Inanition	0	2	22	24
Injury	4	6	3	13
Specific Diseases	0	0	0	0
General infection	0	0	1	1
Tuberculosis	0	0	0	0
Mycosis	0	8	1	9
Parasitic	0	0	2	2
Circulatory	0	0	1	1
Alimentary	1	11	1	13
Respiratory	0	2	9	11
Urinary	0	1	0	1
Reproductive	0	2	3	5
Liver	0	0	0	0
Nervous	0	0	0	0
Endocrine	0	0	0	0
Skin	0	0	0	0
Metabolism and nutrition	1	0	0	1
Neoplasms, etc.	0	0	0	0
Skeleton	0	0	0	0
TOTAL	6	32	43	81

Table 6—Analysis of deaths from mycosis amongst birds during 1958/1960 and January and February 1961.

Total deaths of birds 980
Deaths from mycosis ..... 39=4 per cent.
Total deaths of penguins 24
Deaths of penguins due to mycosis ..... 7=29 per cent.

Species	*Air sacs*	*Lungs*	*Liver*	*Kidneys*	*Nasal passages*	*Alimentary canal*	*Brain*	*Type of lesion*	*Species of fungus*
Hunting Cissa (*Cissa chinensis chinensis*) 79/59	Thoracic & abdominal	Both +	—	—	—	—	—	Plaques and nodes in early stages	
Crimson-crowned Weaver Bird (*Pyromelana hordeacea*) 91/59	Thoracic +	Right +	—	—	—	—	—	Plaques and nodes	
Blue-&-black Tanager (*Tangara braziliensis*) 30/7/58	—	Both +	—	—	—	—	—	Nodes, some calcified	
Purple Sugar Bird (*Cyanerpes caeruleus*) 427/59	Left thoracic +	—	—	—	—	—	—	Plaques	
Streaky-headed Seed-eater (*Poliospiza gularis*) 147/59	—	Both +	—	—	—	—	—	Diffuse, caseous. Mycelium on surface of left lung	
Non-Pareil Bunting (*Passerina ciris*) 449/59	—	Right	—	—	—	—	—	Diffuse caseous	*A. fumigatus*

Table 6 (*continued*)

Species	*Air sacs*	*Lungs*	*Liver*	*Kidneys*	*Nasal passages*	*Alimentary canal*	*Brain*	*Type of lesion*	*Species of fungus*
Red-crested Finch (*Coryephospingus cucullatus*) 419/59	Thoracic +	—	—	—	—	—	—	Plaques	
Malayan Coast White-Eye (*Zosterops palpebrosa aureiventer*) 443/58	—	Left +	—	—	—	—	—	Node caseous	
Resplendent Trogon (*Phalomacrus mocinno*) R152/60	Thoracic and abdominal	Both +	—	—	—	—	—	In air sacs, fruiting mycelium. Lungs diffuse and caseous.	*Aspergillus fumigatus*
Short-billed Humming Bird (*Amazilia versicolor brevirostris*) 601/58	—	—	—	—	—	+ Intestine	—	Nodes	*Aspergillus* sp.
Violet-eared Humming Bird (*Colibri coruscens coruscens* R169/60	Thoracic +	Right ++ Left +	—	—	—	—	—	Plaques in air sacs Nodes in lungs	
Frilled Coquette (*Lophornis magnifica*) 397/58	—	—	—	—	—	+ crop	—	Diffuse growth in food mass	
Mealy Parrot (*Amazona farinosa*) 83/59	Abdominal +	Both +	—	—	—	—	—	Plaques in air sacs. Nodes in lungs	
African Grey Parrot (*Psittacus erithacus*) R177/60	Abdominal +	—	+	—	—	—	—	Fruiting mycelium in air sacs. Diffuse in liver	*A. fumigatus*

African Grey Parrot (*Psittacus erithacus*) R176/60	Abdominal +	—	—	—	—	—	+	Moist and diffuse in air sacs. Diffuse in the brain	
Nicobar Parrakeet (*Psittacula longicauda nicobarica*) 31/59	—	Right +	—	—	—	—	—	One large node	
Red-rumped Parrakeet (*Psephotus haematonotus* 17/11/58	—	Left +	—	—	—	—	—	Single small caseous node	
2 Budgerigars (*Melopsittacus undulatus*) 12/6/58	—	Right lung of one bird +	—	—	—	Intestines +	—	Diffuse in both lungs and guts. (Catarrhal enteritis)	*A. fumigatus*
Cockatiel (*Leptolophus hollandicus*) 17/11/58	—	Both +	—	—	—	—	—	A large node in each lung	
Great Eagle Owl (*Bubo bubo*) 167/58	—	—	+	—	—	—	—	Nodes	
Chilian Flamingo (*Phoenicopterus chilensis*) 670/58	—	Left +	—	—	—	—	—	Superficial plaques	
Black-necked Swan (*Cygnus melancoriphus*) 20/2/61	Abdominal Thoracic +	Both +	—	—	—	—	—	Right abdominal a thickened mass of mycelium. Left abdominal and thoracic plaques. Lungs green nodes	
Common x Chiloe Widgeon (*Anas penelope* x *A. sibilatrix*) 14/11/58	Right abdominal +	Both +	—	Right +	—	—	—	Air sac plaques. Lungs & kidneys nodes	

Table 6 (*continued*)

Species	*Air sacs*	*Lungs*	*Liver*	*Kidneys*	*Nasal passages*	*Alimentary canal*	*Brain*	*Type of lesion*	*Species of fungus*
Ringed Teal (*Anas torquata*) 677/58	Left abdominal + Thoracic +	Both +	—	—	—	—	—	Plaques in air sacs. Nodes in lungs	
Eider Duck (*Somateria mollissima*) 29/10/58	Left abdominal +	—	—	Left +	—	—	—	Small plaque in air sacs. Left kidney destroyed by numerous nodes	
Black-necked Stork (*Xenorhynchus asiaticus*) R122/60	Abdominal and thoracic +	—	—	—	Pharynx +	—	—	Air sacs diffuse and extensive growth of mycelium. Plaques in pharynx	
Northern Guillemot (*Uria aalge aalge*) R59/60	Abdominal +	—	—	—	—	—	—	—	*Aspergillus fumigatus*
Black-footed Penguin (*Spheniscus demersus*) 474/58	Thoracic and abdominal +	+	—	—	—	—	—	Plaques in air sacs. Nodes in lungs	
Black-footed Penguin (*Spheniscus demersus*) 454/59	—	—	—	—	Horny Beak +	—	—	Diffuse growth in horn of beak which showed severe 'hyperkeratosis'.	*A. fumigatus*
Black-footed Penguin (*Spheniscus demersus*) 636/58	—	—	—	—	—	—	+	Diffuse growth in brain, bird suffered from encephalitis	*A. fumigatus*

Black-footed Penguin (*Spheniscus demersus*) 277/60	Right abdominal	—	—	—	—	—	—	Diffuse mycelial growth	
Black-footed Penguin (*Spheniscus demersus*) 129/59	Thoracic and abdominal +	+	—	—	—	—	—	Diffuse mycelial growth in air sacs. Plaques in lungs	
Humboldt's Penguin (*Spheniscus humboldti*) R369/60	Thoracic +	Both +	—	Both +	—	—	—	Diffuse mycelium in air sacs. Nodes in both lungs. Left kidney destroyed by nodes. Right kidney also nodes	*A. fumigatus*
Magellan Penguin (*Spheniscus magellanicus*) 114/58	Thoracic +	Both +	—	Both +	—	—	—	Diffuse mycelial growth in air sacs. A few nodes in lungs. Left kidney destroyed by nodes. Right kidney also nodes	*A. fumigatus*
Southern White-breasted Crake (*Laterallus leucopyrrhus*) R200/59	Abdominal +	Both +	—	—	—	—	—	Plaques in air sacs. Nodes in lungs	
Wonga Wonga Pigeon (*Leucosarcia melanoleuca*) 209/59	Thoracic and abdominal +	Both +	—	—	—	—	—	Plaques in air sacs and lungs. Liver and lungs also affected with numerous tubercles, as a result of 'tuberculosis'	

Table 6 (*continued*)

Species	*Air sacs*	*Lungs*	*Liver*	*Kidneys*	*Nasal passages*	*Alimentary canal*	*Brain*	*Type of lesion*	*Species of fungus*
Harlequin Quail (*Coturnix d. delegorguei*) 269/59	—	—	—	—	+	—	—	Nasal cavities and sinuses filled with a caseous mass containing mycelium	
White-crested Guan (*Pipile jacutinga*) 460/59	Thoracic and abdominal +	—	—	—	—	—	—	Plaques	*A. fumigatus*

THE EMBRYONIC MEMBRANES AND PLACENTATION IN FIVE GENERA OF DIPROTODONT MARSUPIALS

BY

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(With 4 plates and 4 figures in the text)

The embryonic membranes of the diprotodont marsupials *Trichosurus vulpecula*, *Pseudocheirus peregrinus* (Phalangeridae) *Protemnodon rufogrisea*, *Setonix brachyurus* and *Potorous tridactylus* (Macropodidae) are described.

Three egg membranes—zona pellucida, albumen layer and shell membrane—are present. The shell membrane persists for almost the entire gestation period (17·5 days) in *Trichosurus* and for about 20 days in *Setonix* which has a gestation period of 27 days.

All species have yolk-sac placentation and in none does the allantois take part in the formation of a placenta. The bilaminar omphalopleure (non-vascular yolk-sac placenta) appears early and the vascular omphalopleure comparatively late in uterine development.

The amnion and allantois appear comparatively late in pregnancy. In *Setonix* amnion folds are not evident at 17 days and are incompletely developed at 19 days. No allantoic primordium may be recognised in *Setonix* at 19 days. The allantois is however of comparatively large size in near term embryos of *Pseudocheirus*, *Protemnodon* and *Setonix*. In *Pseudocheirus* the allantois reaches almost to the chorion but in *Protemnodon* and *Setonix* it is excluded from the chorion by the great development of the yolk-sac splanchnopleure.

The early embryos of all species are orientated with the head towards the anterior end of the uterus but late embryos of *Trichosurus*, *Pseudocheirus*, *Protemnodon* and *Setonix* have the head towards the posterior end of the uterus. Late embryos of *Potorous* have not been studied. In all species the bilaminar omphalopleure is apposed to the anterior part of the uterine wall and separated from the posteriorly placed vascular omphalopleure by the sinus terminalis which follows an equatorial or oblique course around the inner surface of the uterine wall. A small area of true chorion is apposed to the uterine wall near the uterine neck.

In all species the uterine lumen epithelium remains complete throughout pregnancy. Embryonic and maternal blood streams are separated by the two endothelia, by embryonic mesenchyme and maternal connective tissue and by embryonic ectoderm and maternal epithelium. The uterine endometrium is well vascularised in *Trichosurus* and *Pseudocheirus* and in the latter embryonic and maternal blood streams are brought into close proximity by displacement of nuclei from the cell layers separating the blood vessels. In the remaining species (all macropod marsupials) the uteri are relatively avascular and embryonic and maternal blood streams are well separated.

Material giving a positive periodic acid-Schiff reaction is found in the uterine glands and appears to serve an embryotrophic function. Further P.A.S. positive material, found within the embryonic membranes, gives positive tests for glycogen. In *Protemnodon* a considerable amount of material, consisting of degenerating cells, apparently serves a histotrophic function. The vascular omphalopleure appears to be the main organ of embryotrophic nutrition in the closing stages of pregnancy.

It is suggested that yolk-sac placentation is the primitive marsupial condition and that allantoic placentation has been evolved, within the group, in at least two separate lines.

* Present address.

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INTRODUCTION

Owen (1834, 1837) examined two intra-uterine embryos of the kangaroo, *Macropus major*, and observed that the well vascularised yolk-sac was in intimate association with the maternal uterine epithelium. In the larger of the two embryos he identified the allantois which did not reach the chorion. These observations were confirmed by Chapman (1882) who examined a single embryo, of the same species, of comparable size to the largest embryo examined by Owen. A similar arrangement of embryonic membranes was found in the majority of marsupials subsequently investigated. These include *Didelphis virginiana* (Selenka, 1887) and a variety of phalangers and macropod marsupials (Selenka, 1892 ; Semon, 1894 ; Hill, 1895, 1901 ; Flynn, 1930 ; Sharman, 1955 b). In these the membranes apposed to the uterine wall consist of the vascular and non-vascular portions of the yolk-sac and a small area of true chorion consisting of embryonic ectoderm and somatic mesoderm. The allantois, which may not appear until comparatively late in pregnancy, remains buried in folds of the yolk-sac splanchnopleure and does not reach the chorion.

In *Dasyurus quoll* (=*D. viverrinus*) there is a well developed yolk-sac placenta and the allantois becomes apposed to the chorion. According to Hill (1900) the allantoic vessels are degenerate and as development proceeds the allantois retreats from the chorion without taking part in the formation of a placenta. The allantois of *Phascolarctos cinereus* also becomes apposed to

the chorion but according to Caldwell (1884) and Semon (1894) its vessels do not vascularise a placenta. Semon attributed a respiratory function to the allantois of this species. Amoroso (1952) stated that allantoic placentation occurs in *Phascolarctos* and also in *Phascolomis* but gave no details. The existence of allantoic placentation in these two marsupials might also be inferred from the statement by Hill (1949) that " he now regarded the simple apposed chorio-allantoic-omphalopleural placenta as seen in *Phascolarctos* and *Phascolomis* as the most primitive type amongst the Marsupialia . . . . " Caldwell's (1884) brief description of the embryonic membranes of *Protemnodon rufogrisea* (=*Halmaturus ruficollis*) has been interpreted by several authors (e.g. Jenkinson, 1913 ; Flynn, 1930 ; Pearson, 1947) to mean that this species has a similar arrangement of embryonic membranes to that found in *Phascolarctos*. The arrangement is however similar to that found in other macropod marsupials (Hill, 1895). New material has been studied and is described in this paper.

In at least three species of the marsupial family Peramelidae (*Perameles nasuta, P. gunnii* and *Isoodon obesulus* (=*P. obesula*) a true chorio-allantoic placenta is present as well as the yolk-sac (chorio-vitelline) placenta (Hill, 1898, 1901). The allantoic placenta is a complex syncytial structure of mixed maternal and embryonic elements (Flynn, 1923 b ; Hill, 1932) which Amoroso (1952) designated a " placenta endothelio-endothelialis ".

In the marsupials *Dasyurus* (Hill & O'Donoghue, 1913), *Didelphis* (Hartman, 1923) and *Setonix* (Sharman, 1955 a, b) post-ovulatory changes in the reproductive organs of non-pregnant females are essentially similar to those occurring in pregnant females. No marsupial is known in which the gestation period exceeds the length of one oestrous cycle (Sharman, 1959) and it has been suggested that the marsupial placenta may not have evolved those endocrine functions which, in the Eutheria, supplement the actions of ovarian and pituitary hormones and allow the embryo to be retained in the uterus beyond the limits of the oestrous cycle (Amoroso, 1955). In *Didelphis* ovariectomy, performed at any stage of pregnancy, results in abortion or resorption of the uterine embryos (Hartman, 1925) but in many eutheria pregnancy proceeds normally if the ovaries are removed after the initial stages. The effect of ablation of the corpus luteum and ovariectomy on the course of pregnancy in *Trichosurus* is currently being studied.

The embryonic membranes of the following marsupials are described in this paper. The classification is that of Simpson (1945).

PHALANGERIDAE

Phalangerinae

Trichosurus vulpecula Kerr—the brush-tailed possum. Observations on reproduction in this species were made by Lyne, Pilton and Sharman (1959). The female is polyoestrous and the length of the oestrous cycle averages 24·5 days. Pregnancy lasts about 17·5 days and but one embryo is borne at a time (Pl. 2, fig. 7). Buchanan and Fraser (1918) and Fraser (1919) described various aspects of the embryology of *Trichosurus*. Hill (1901) stated that

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Trichosurus had only yolk-sac placentation but did not describe the embryonic membranes.

Phascolarctinae

Pseudocheirus peregrinus Boddaert—the ring-tailed possum. In his book on the mammals of South Australia Jones (1924) referred to this species as *P. laniginosus*, but, according to Tate (1945), *peregrinus* and *laniginosus* are conspecific so the prior name is used here. The female *Pseudocheirus*, unlike the other species here treated, is polytocous. No accurate data is available about breeding cycles except that the female is polyoestrous, with an oestrous cycle of about 28 days length (Phyllis E. Pilton, unpublished). The length of the gestation period is unknown. Some brief observations on the placenta of the related marsupial *P. convolutor* (*P. cooki*) were made by Flynn (1922).

MACROPODIDAE

Macropodinae

Protemnodon rufogrisea frutica (Ogilby)—Bennett's wallaby. Very little is known about reproductive processes in this marsupial. The gestation period was stated to be 40 days (Jennison, 1927). The occurrence of a retrogressing corpus luteum, in addition to the corpus luteum of pregnancy, in the ovaries of one of the specimens (Pr2) studied in this investigation indicates that the female is polyoestrous. A brief description of the embryonic membranes was given by Caldwell (1884). Hill (1895) stated that the yolk-sac placenta was typically like that of other macropod marsupials.

Setonix brachyurus (Quoy and Gaimard)—the quokka. This species has been bred in captivity (Waring, Sharman, Lovat & Kahan, 1955). In the non-lactating domestic female, successive oestrous periods occur throughout the year at intervals of approximately 28 days. In the wild state the breeding season extends from February to about August each year (Sharman, 1955 a). Pregnancy lasts about 27 days but the uterine luteal phase, which is presumably maintained by the secretion of progesterone from the corpus luteum (Sharman, 1955 a), lasts no longer in the pregnant female than it does in the non-pregnant female (Sharman, 1955 b). If the occurrence of a uterine luteal phase be accepted as a criterion of progesterone secretion then pregnancy outlasts the period of functional activity of the corpus luteum in *Setonix* by six to seven days. Parturition is followed by a post-partum oestrus at which the female will mate and become fertilized. The resulting embryo does not however develop beyond a unilaminar blastocyst stage while the young resulting from the earlier pregnancy is suckled in the pouch. Use has been made of this lactation controlled delayed implantation to obtain a series of approximately dated embryos for this study. Removal of the suckling young from the pouch causes cessation of lactation with subsequent resumption of development by the quiescent blastocyst. A second birth occurs about 25 days after removal of the pouch young (H. Tyndale-Biscoe, pers. comm.). Females killed before this time have a uterine embryo of approximately known degree of development (Sharman, 1957).

Potoroinae

Potorous tridactylus (Kerr)—the potoroo. *Potorous* is monovular and polyoestrous and has a gestation period of about six weeks (Flynn, 1930). R. L. Hughes (pers. comm.) has shown the period between successive ovulations to average 42 days in the non-lactating female, and the time from effective copulation to birth to be 38 days. A lactation controlled delayed implantation, like that in *Setonix*, occurs. The embryonic membranes have not previously been described.

MATERIAL AND METHODS

A detailed list of the material studied is given in the appendix. The embryos of *Pseudocheirus*, *Protemnodon* and *Potorous* were taken from females collected in the wild so are of unknown age. The embryos of *Trichosurus*, excepting the 4-cell stage (T1), were taken from females at known times after mating or were aged by comparison with embryos of known age. The embryos of *Setonix* of known age were obtained by killing females, kept in captivity, at various intervals after observed copulation or after the first appearance of sperms in the daily vaginal smear. Six embryos (numbers S5, S7, S8, S9, S11, and S12) were obtained by killing females, which had been carrying delayed unimplanted blastocysts, at various intervals after removal of the pouch young (see p. 200). The ages given for these embryos (appendix) are thus the equivalent ages of embryos derived from normal mating.

Reproductive systems from pregnant females were fixed entire in Bouin's, Bodian's or Rossman's fluid. Uteri containing early embryos were serial sectioned without prior dissection but those containing later embryos were dissected before embedding. No uterus contained more than one embryo which was always located at the posterior end. A considerable amount of the anterior end of each uterus could therefore be dissected away without damaging the embryo although much of the bilaminar omphalopleure was removed in this process (Pl. 1, figs. 2–5).

Dissected uteri were orientated during embedding so that longitudinal sections of the embryo could be cut (Pl. 2, figs. 6, 8, 9). Pregnant uteri were cut in serial section at thicknesses ranging from 6 to 15 microns. Routine staining was Ehrlich's haematoxylin and eosin. Histochemical tests were made according to the methods of Pearse (1953). The embryos were measured in longitudinal section after staining. Only one measurement, greatest length (G.L.), was taken.

REPRODUCTIVE SYSTEMS

The reproductive systems of all species examined conform to the basic marsupial pattern. The uteri are completely separate and open into the anterior vaginal chamber on separate papillae. *Pseudocheirus* bears two or three young so embryos are found in both uteri but in the other (monotocous) species one uterus alone carries an embryo during pregnancy (Pl. 1, fig. 1).

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The lateral vaginal canals unite posteriorly and open, together with the urethra, into the urogenital sinus. Birth in *Trichosurus* occurs by way of the

median vaginal culs-de-sac and a short pseudo-vaginal canal which closes after each parturition (Pearson, 1945; Sharman, 1954). In *Pseudocheirus* the vaginal culs-de-sac are not as well developed as in *Trichosurus* and a long pseudo-vaginal canal opens at each parturition (Pearson & de Bavay, 1959; Phyllis E. Pilton unpublished). In *Setonix* the pseudo-vaginal canal, once formed, remains permanently open (Waring, *et al.*, 1955). Direct communication between the median vaginal culs-de-sac and urogenital sinus occurs in parous females of *Protemnodon rufogrisea* (=*Halmaturus ruficollis*). In non-parous females direct communication does not usually exist but one virgin female was found in which it was in process of formation (Fletcher, 1883). *Potorous* differs from other marsupials in that birth occurs by way of the lateral vaginal canals (Flynn, 1923 a). Pearson (1945) stated that this mode of birth " is to be regarded not as a primitive characteristic but as a secondary reversion to a prototypal arrangement".

EGG MEMBRANES

The early embryo is surrounded by three egg membranes—zona pellucida, albumen layer and shell membrane (Fig. 1). The zona pellucida and albumen layer are short lived but the shell membrane is present until at least some mesoderm is extended beyond the embryonal area.

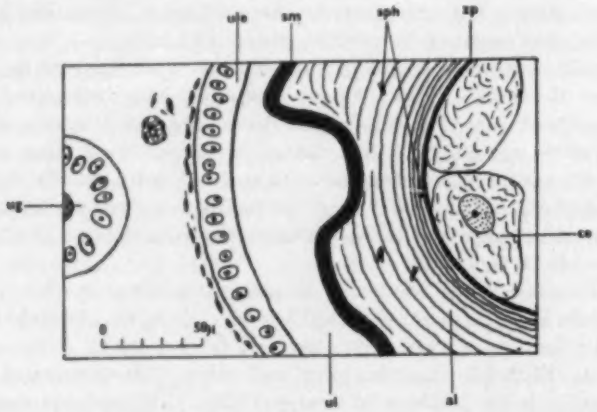

Fig. 1—Part of section of a 4-cell stage of *Trichosurus vulpecula* (T1), with adjacent uterine mucosa, showing egg membranes.

al, albumen layer; ce, cell; sm, shell membrane; sph, sperm heads; ug, uterine gland; ul, uterine lumen; ule, uterine lumen epithelium; zp, zona pellucida.

(a) *Zona pellucida*

This is present in a fertilized tubal egg of *Setonix* (S1) previously described (Sharman, 1955 b). In a 4-cell stage from the uterus of *Trichosurus* (T1) the zona pellucida has a thickness of about 0·001 mm. (Fig. 1). In both tubal egg and 4-cell stage spermatozoa are embedded in the zona pellucida and in the overlying albumen layer. The zona pellucida cannot be recognized with

certainty in later stages but is probably present in the unilaminar blastocyst of *Setonix* (S2).

(b) *Albumen layer*

In the tubal egg the albumen layer varies in thickness from 0·006 to 0·016 mm. It is much thicker in the 4-cell stage. In a unilaminar blastocyst of *Setonix* (S2) it is 0·005 to 0·007 mm. in thickness. This blastocyst is equivalent in stage of development to the quiescent blastocyst carried during delayed implantation in *Setonix* (Sharman, 1955 b). The albumen layer cannot be recognized in later stages.

(c) *Shell membrane*

The shell membrane was recognized in marsupial eggs by Caldwell (1887) and by Hill (1910) who regarded it as homologous with the shell of the monotreme egg. The monotreme egg shell is laid down in three parts—the basal layer, the rodlet layer and the external layer. According to Hill (1933) the shell of the marsupial egg does not advance beyond the basal layer of the monotreme shell. As pointed out by Hill (1910) the shell membrane of marsupials is distinguishable at once from the albumen layer by virtue of its optical characters and staining reactions. A shell membrane, which breaks down early in development, is present in viviparous reptiles (Weekes, 1930).

The shell membrane is possibly represented in the tubal egg by a very thin layer external to the albumen layer. In the 4-cell stage it is approximately 0·004 mm. in thickness. It shows remarkable persistence in later embryos. It forms a complete covering 0·004 mm. thick to the unilaminar blastocyst and is present in all later stages except the near term embryos of *Setonix* and *Pseudocheirus* and the two embryos of *Protemnodon*. Thus in the 19 day *Setonix* (S8) it surrounds the entire embryo and separates the embryonic membranes from the uterine epithelium (Pl. 4, fig. 17). In another 19 day embryo (S7) the shell membrane has degenerated and it shows signs of breaking down in an 18 day embryo (S6). It is absent in a 21 day embryo but remnants occur in a 23 day embryo. Remnants of the shell membrane persist in the 16·5 day embryo of *Trichosurus* (T5, one day before birth). In breaking down the shell membrane first becomes incomplete at the anterior end of the uterus in the region of the bilaminar omphalopleure. It persists longer in the region of the vascular omphalopleure and still longer in region of the chorion. As it breaks down its fragments collect in the lower part of the uterus.

EMBRYONIC MEMBRANES

(a) *Trophoblast*

No inner cell mass is present in marsupials and the trophoblast, as well as producing ectodermal derivatives, gives rise to all other embryonic and extra-embryonic tissues. For this reason the name protoderm has been proposed for the unilaminar layer constituting the marsupial blastocyst (see Boyd & Hamilton, 1952). In the only unilaminar blastocyst studied (*Setonix*, S2) the trophoblast consists of a layer of thin attenuated cells of about 0·006 mm.

thickness, measured across the nuclei. The trophoblast persists in all species as an outer layer of extra embryonic ectoderm apposed to the uterine epithelium. Throughout development the nuclei of this layer stain more deeply with haematoxylin than do the nuclei of the extra-embryonic endoderm.

(b) *Bilaminar omphalopleure*

Primitive endodermal cells appear in blastocysts of the marsupial, *Dasyurus*, measuring 4·5 to 5·0 mm. diameter (Hill, 1910). The endoderm subsequently extends around the inner surface of the unilaminar blastocyst converting it to a bilaminar blastocyst. The wall of the yolk-sac thus formed, consisting of ectoderm and endoderm, is of considerable importance as a placental structure and for this Hill (1898) proposed the name bilaminar omphalopleure.

The bilaminar omphalopleure is the first of the extra-embryonic membranes to form a close association with the maternal uterine epithelium. In the earliest embryos the ectoderm and endoderm cells constituting this membrane are thin and flattened (Pl. 3, fig. 10) but as development proceeds they enlarge and come to exceed in size their counterparts in the vascular omphalopleure (Pl. 3, compare figs. 11 and 13).

(c) *Vascular omphalopleure*

In the 12·5 day embryo of *Trichosurus* the mesoderm hardly extends beyond the embryonal area. In slightly more advanced embryos of *Setonix* (S4) and *Pseudocheirus* (P1), both of unknown age, the mesoderm is extended between ectoderm and endoderm of the embryonic membranes to form a small area of vascular omphalopleure. In the 17 day embryo of *Setonix* (S5) the vascular omphalopleure is also confined to a small area. In later embryos it is better developed and forms a broad band around the adembryonic hemisphere. The vascular omphalopleure appears to constitute the main organ for physiological exchange, between mother and embryo, in all late stages studied.

(d) *Chorion*

The term chorion is here restricted to that portion of the extra-embryonic membranes which consists only of ectoderm and somatic (non-vascular) mesoderm. In all species studied the chorion is apposed to a posterior portion of the uterine wall near the opening of the uterine neck (Fig. 4 ; Pl. 2, figs. 8, 9). Early in development the cells of both layers of the chorion are thin and flattened. In *Trichosurus*, which has a short gestation period, they remain in this condition throughout uterine development but in *Setonix* they become large in the latter stages of pregnancy (Pl. 4, fig. 21).

(e) *Amnion and pro-amnion*

The amnion arises by fold formation and appears comparatively late in development ; in *Setonix* amnion folds cannot be distinguished in the 17 day embryo (S5, G.L. 4·0 mm). In *Pseudocheirus*, head and tail amnion folds are discernible in embryo P1, G.L. 5·4 mm., age unknown. In the 19 day embryo of *Setonix* (S8, G.L. 4·75 mm.) the amnion folds are well developed but do not meet above the body in the mid-region. A chorio-amniotic connection,

or a remnant of this connection, is found in later embryos (Fig. 2 ; Pl. 2, fig. 8), but is not present in near term embryos. In the Sauropsida and Monotremata the chorio-(sero-) amniotic connection usually persists throughout incubation (Boyd & Hamilton, 1952).

An extensive pro-amnion is present after fold formation is complete (Fig. 2 ; Pl. 1, fig. 4 ; Pl. 2, fig. 8) but as development proceeds this is progressively reduced by extension of mesoderm between the layers of ectoderm and endoderm constituting the pro-amnion. In *Setonix* the pro-amnion is reduced to a remnant at the anterior end of the 21 day embryo and is absent in later stages. The time at which it disappears is unknown for the other species but in none does it persist throughout gestation as it does in the marsupials *Didelphis virginiana* and *Dasyurus quoll* (see Amoroso, 1952).

(f) *Allantois*

The allantois appears comparatively late in development. It is small in the 16·5 day embryo of *Trichosurus* (T5). No allantoic primordium may be recognized in the 19 day embryo of *Setonix* (S8). The allantois is however of comparatively large size in the 25 day *Setonix* (Pl. 1, fig. 3) and still larger in a late embryo of *Pseudocheirus* (Pl. 1, fig. 5). The volume of the allantois in this embryo (calculated from measurements on serial sections) is about 2·5 cc.—that of the embryo 2·75 cc. It is frequently stated, and indeed currently assumed, that the allantois is small in all marsupials. This may be true as far as its actual dimensions are concerned but its size, compared with that of the embryo, is in fact large in late stages of uterine development in those marsupials which have comparatively long gestation periods. This is not unexpected since, in none of the species studied here, does the allantois reach the chorion. No mechanism therefore exists for the transfer of the contents of the allantois to the maternal blood stream and these must be retained until parturition.

(g) *Arrangement of embryonic membranes*

In the marsupials *Didelphis virginiana* (Hartman, 1923) and *Setonix brachyurus* (Sharman, 1955 a, b) the fertilized, or unfertilized, egg reaches the uterus one day after ovulation. Fertilized, but unsegmented, ova were found in the uterus of *Dasyurus quoll* (Hill, 1910) and 2-cell stages in the uterus of *Didelphis aurita* (Hill, 1918). By contrast segmenting eggs are found in the Fallopian tubes of eutherian mammals for up to seven days after ovulation (Boyd & Hamilton, 1952) and morula stages of 28 or more cells are found in the Fallopian tubes of the cat (Hill & Tribe, 1924). The data obtained in this investigation indicates that in *Trichosurus*, like in other marsupials, the egg reaches the uterus soon after ovulation. In specimen T1 a 4-cell stage was found in the lower part of the uterus 5/8 of the distance from the opening of the Fallopian tube to the os uterus. All early embryonic stages occurred in a comparable position so it is probable that implantation takes place at this point, the uterus thereafter being progressively filled by an extension of embryonic and extra-embryonic tissues.

After formation of the vascular omphalopleure the membranes apposed to the uterus consist of the anteriorly placed bilaminar omphalopleure

separated from the more posterior vascular omphalopleure by the sinus terminalis which follows an equatorial or oblique course around the inner surface of the uterine wall (Pl. 1, fig. 3 ; Pl. 2, fig. 8). After fusion of the amnion folds a small area of true chorion is apposed to the posterior portion of

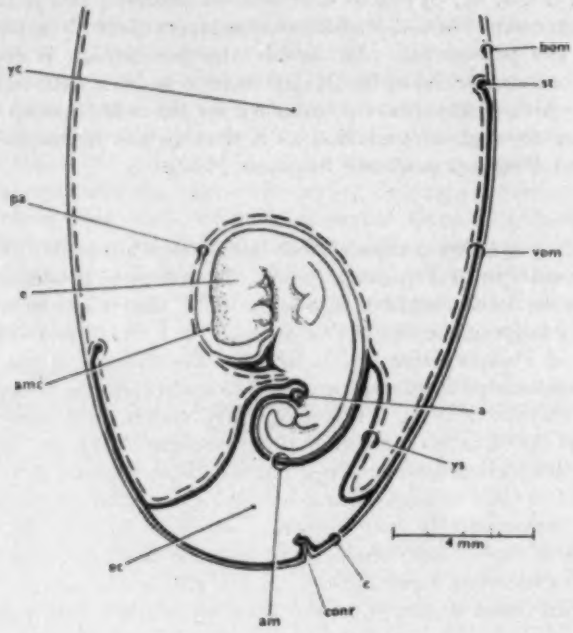

Fig. 2—The arrangement of the embryonic membranes, early stage, in *Trichosurus vulpecula*, *Protemnodon rufogrisea*, *Setonix brachyurus* and *Potorous tridactylus*. Thin line ectoderm, thick line mesoderm, broken line endoderm.

a, allantois ; am, amnion ; amc, amnion cavity ; bom, bilaminar omphalopleure ; c, chorion ; conr, remnant of chorio-amniotic connection ; e, embryo ; ec, extra-embryonic coelom ; pa, pro-amnion ; st, sinus terminalis ; vom, vascular (trilaminar) omphalopleure ; yc, yolk-sac cavity ; ys, yolk-sac splanchnopleure.

the uterine mucosa (Fig. 2). Later changes do not affect the disposition of these membranes. As development proceeds the embryo, and its allantois, become to some extent invaginated in folds of the yolk-sac splanchnopleure (Figs. 3, 4). This invagination is complete in *Setonix*, not so complete in *Trichosurus* and slight in *Pseudocheirus* where the allantois reaches almost to the chorion. In the 25 day embryo of *Setonix* (S12) and the late embryo of *Protemnodon* (Pr2) the folds of the yolk-sac splanchnopleure, after passing from the umbilicus around the body of the embryo, fuse together before reaching the uterine wall. This has the effect of enclosing the embryo in a second "amnion" which also encloses the allantois. In late stages the embryo, hitherto orientated with the head towards the anterior end of the uterus (Fig. 2)

faces posteriorly (Figs. 3, 4). Turning occurs at about 20 to 21 days in *Setonix* and at about 15 days in *Trichosurus*.

The arrangement of the blood vessels supplying the vascular omphalopleure in late stages of pregnancy differs in no essential way from the arrangement described by Hill (1901) for *Protemnodon parma* (=*Macropus parma*). The vitelline artery divides into unequal sized branches before it leaves the yolk-stalk. Both branches give off fine vessels to the yolk-sac splanchnopleure and the larger branch, before it divides on the uterine wall to form the sinus

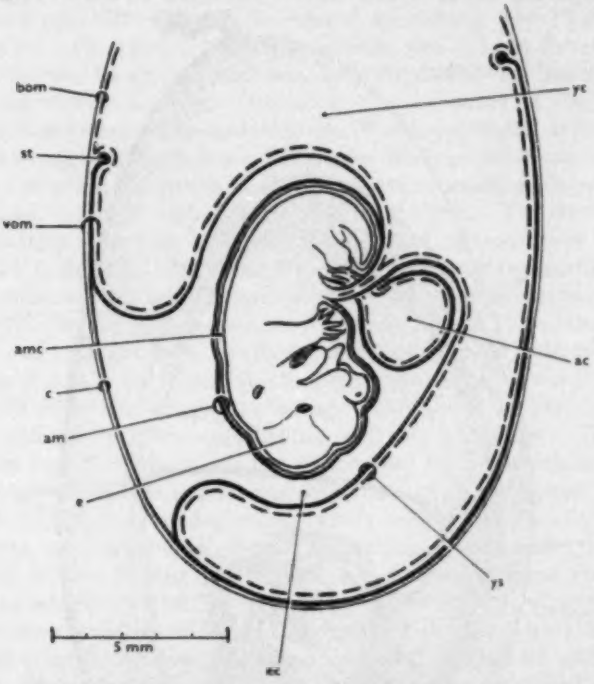

Fig. 3—The arrangement of the embryonic membranes in *Trichosurus vulpecula* (16·5 days) and *Setonix brachyurus* (21 days). The arrangement is similar to that found in late stages of pregnancy in *Setonix brachyurus* and *Protemnodon rufogrisea* except that in these the allantois is larger and the embryo is completely enshrouded by folds of the yolk-sac splanchnopleure. In late stages of *Pseudocheirus peregrinus* the allantois is large and passes round one side of the embryo so as to reach almost to the chorion.
ac, allantoic cavity. Other letters as in Fig. 2.

terminalis, supplies part of the vascular omphalopleure. The sinus terminalis is continuous around the diameter of the uterus being completed, opposite where the vitelline artery divides, by an anastomosis. The blood returning from the placenta is collected by four large veins which pass separately into the yolk-sac splanchnopleure, two on either side of the embryo. Within the splanchnopleure the veins on either side of the embryo unite together and the two veins thus formed pass separately towards the body of the embryo and unite into a single vitelline vein within the yolk stalk (Fig. 4).

PLACENTAL STRUCTURE

(a) *Trichosurus vulpecula*

The cells of the lumen epithelium of the uterus containing a 15 day embryo (T4) are of uniform size and have pale staining nuclei placed at regular intervals. Many of the epithelial cells have conspicuous vacuoles (Pl. 3, fig. 10). The subepithelial capillary layer is conspicuous and the entire uterine mucosa is well vascularised. Polymorphonuclear leucocytes, as well as red blood

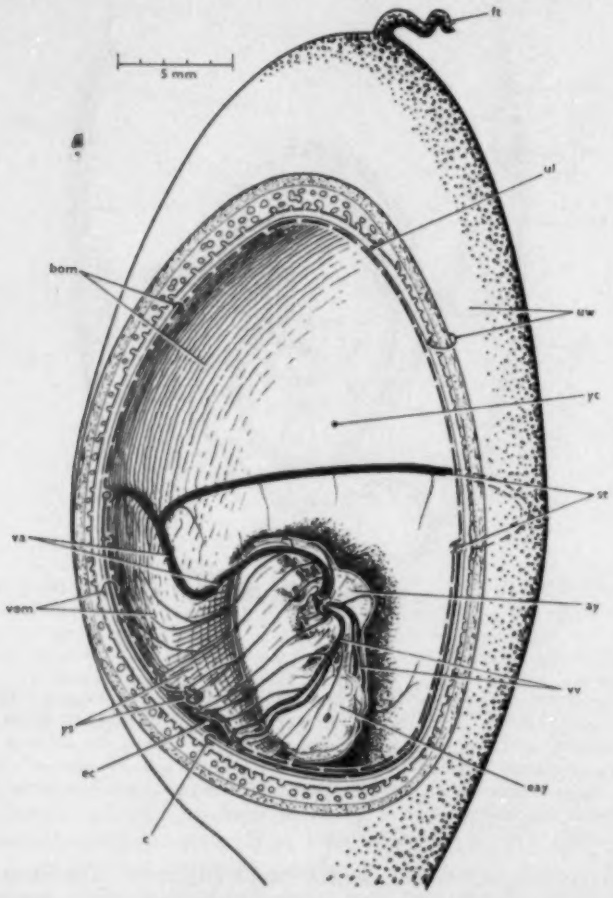

Fig. 4—Diagrammatic representation of the embryo and its relationships to the uterus, based on a 16·5 day embryo of *Trichosurus vulpecula* (T5). Part of the uterine wall and membranes cut away. In section the ectoderm is shown as a thin line, the mesoderm as a thick line and the endoderm as a broken line.

ay, allantois, beneath yolk-sac splanchnopleure; eay embryo, beneath amnion and yolk-sac splanchnopleure; ft, Fallopian tube; ul, uterine lumen; uw, uterine wall (mucosa and muscularis); va, vitelline artery; vv, vitelline vein. Other letters as in Fig. 2.

corpuscles, occur in the maternal vessels. Degenerating cells are found in a few places free in the uterine lumen between the bilaminar omphalopleure and maternal epithelium. None are observed in the regions of the vascular omphalopleure and chorion. Material giving a positive McManus periodic acid-Schiff (P.A.S.) reaction occurs in the lumina of uterine glands and uterus. Its distribution is more fully discussed in the description of the placenta of the later embryo of *Trichosurus*.

The maternal epithelium of the uterus containing the 16·5 day embryo is complete and resembles that of the uterus containing the 15 day embryo except that its cells are of a little less uniform size. As in the earlier stage polymorphonuclear leucocytes occur in the sub-epithelial capillaries and deeper vessels of the uterine mucosa. Occasional mitoses occur in the endothelial cells of the capillaries. The endothelium of the capillaries is undoubtedly present and is presumably separated from the uterine epithelium by maternal connective tissue but the nuclei of this layer are squeezed aside so that none are interposed between endothelium and epithelium. The remaining cell layers separating maternal and embryonic blood streams may be readily identified (Pl. 3, fig. 12). These are the extra-embryonic ectoderm, a layer of embryonic mesenchyme, and the endothelium of the embryonic capillaries.

Where the embryonic membranes are separated from the maternal epithelium material giving a positive P.A.S. reaction is collected. This is derived from P.A.S. positive material found in the uterine glands (Pl. 3, fig. 10) which exudes from the gland mouths into the uterine lumen but material secreted by cells of the uterine epithelium may also contribute. The material in the glands and that separating the membranes from the epithelium is not digested by saliva nor does it give a positive reaction for glycogen with Best's carmine. P.A.S. positive material also occurs inside the cells of the vascular and bilaminar omphalopleures, chorion and uterine epithelium ; as droplets in the vessels of the yolk-sac splanchnopleure ; sparingly in the vessels of the vascular omphalopleure and on the inner surface of the chorion—that is within the extra-embryonic coelom. This material, though of wide distribution, is much less in quantity than the P.A.S. positive material of uterine glands and lumen. It is removed by digestion with saliva and gives a positive reaction with Best's carmine so is presumably glycogen.

(b) *Pseudocheirus peregrinus*

The bilaminar omphalopleure, in the earliest of the two embryos, consists of two layers of flattened cells. Mesoderm is extended between ecotodermal and endodermal layers in a small portion of the extra-embryonal area converting part of the yolk-sac wall to a vascular omphalopleure. Amnion formation is restricted to very small head and tail folds (Pl. 2, fig. 6). The entire blastocyst is surrounded by a shell membrane. The uterine epithelium consists of a single layer of cells and the subepithelial capillary layer is but poorly represented. Few vessels occur in the uterine mucosa.

In the late embryo the bilaminar omphalopleure consists, over much of its surface, of two layers of cells of uniform size. In certain regions, opposite blood vessels in the uterine mucosa, the ectodermal cells are larger and have

giant nuclei. These large ectodermal cells closely follow the contours of the uterine mucosa and are separated from the uterine epithelium by a thin layer of P.A.S. positive substance. In these areas the nuclei of the epithelial cells are squeezed aside and the embryonic membrane is separated from the maternal blood stream by the flattened epithelial cells, maternal connective tissue which is also exceedingly flattened, and the endothelial wall. Elsewhere in the region of the bilaminar omphalopleure nuclei are regularly placed along the uterine epithelium. The P.A.S. positive material separating the uterine epithelium from the large ectodermal cells differs in no way in its staining reactions from P.A.S. positive material which exudes into the uterine lumen from the mouths of the uterine glands yet its location, in some cases at least, indicates that it may be derived, not from the glands, but from the underlying maternal tissues. This material, and the material which is formed in the glands and exudes into the uterine lumen, is not digested by saliva nor does it give a positive reaction with Best's carmine. P.A.S. positive material which is digested by saliva, and which gives a positive test with Best's carmine is however found within the endodermal cells of the bilaminar omphalopleure.

In the region of the vascular omphalopleure there is a close apposition between embryonic membranes and the maternal uterine epithelium. The extra-embryonic ectoderm consists of cells of irregular shape with large, well-spaced, nuclei separated by scant cytoplasm. The embryonic mesenchyme and endothelial cells are stretched thinly between their nuclei so that the embryonic red blood corpuscles, though covered by three cell layers, are brought very close to the surface of the embryonic membrane (Pl. 3, fig. 14). The maternal uterine epithelium is present but its nuclei, instead of being evenly spaced, are often absent from regions overlying sub-epithelial capillaries and are grouped together elsewhere. The underlying maternal connective tissue is likewise very thin and nuclei are largely absent from the maternal endothelium on the inner surface of the sub-epithelial capillaries. The net result is that, although embryonic and maternal blood streams are separated by six layers of cells, they are spatially very close (Pl. 3, fig. 15). A feature of the vascular yolk-sac placenta, but poorly developed in *Trichosurus*, is the regular occurrence along the inner side of the uterine mucosa of projections into the lumen containing abundant capillaries but no glands. In these regions there is very close apposition of foetal and maternal tissues (Pl. 4, fig. 16). In the same regions nuclei of embryonic mesoderm cells, not apparently separated by cell walls, occur in clusters. As in the region of the bilaminar omphalopleure, P.A.S. positive material is produced in the glands and exudes from the gland mouths into the uterine lumen. P.A.S. positive material is also produced at the surface of the uterine mucosa and forms, in many places, a thin layer separating the vascular omphalopleure from the mucosa, especially in those areas where groups of sub-surface capillaries project into the lumen. None of the P.A.S. positive material within the uterine glands nor any of that occurring outside the embryo and its membranes is digested by saliva. Further P.A.S. positive material, found in small quantity inside the ectoderm and endoderm cells of the vascular omphalopleure and as droplets

within the allantoic vein where it enters the yolk stalk, is digested by saliva and gives a positive reaction with Best's carmine.

The extra-embryonic ectoderm cells of the chorion are large and have near spherical nuclei, the somatic mesoderm cells of the same region are thin and flattened. Uterine glands near the region of the chorion have abundant P.A.S. positive material and this exudes from the mouths of the glands in great quantity filling such spaces as occur between the chorion and uterine epithelium. Even where membranes and epithelium are closely placed a thin layer of P.A.S. positive material is interposed. This material is other than glycogen but intra-cellular P.A.S. positive material, occurring sparsely in both ectoderm and mesoderm of the chorion, gives positive histochemical tests for glycogen.

The subepithelial capillary layer consists of numerous vessels which are much larger than those of the uterus containing the earlier embryo. The entire mucosa is exceedingly vascular and mitoses commonly occur in the endothelial cells of both sub-epithelial capillaries and deeper vessels. Occasional leucocytes occur in the blood vessels but more commonly these are found within the connective tissue of the mucosa. In some regions, especially in those associated with the bilaminar omphalopleure, leucocytes appear in the spaces separating maternal epithelium and embryonic membranes. These are apparently of histotrophic significance for they are found, in various stages of disintegration, within the membranes.

(c) *Protemnodon rufogrisea*

In the earliest of the two embryos (Pr1) P.A.S. positive material is found within the cavities of the uterine glands. This is infrequent in the glands at the anterior end and more frequent in those at the posterior end of the uterus. It is therefore more frequently expelled into the uterus from the gland mouths in the regions of the vascular omphalopleure and chorion and it is in these regions that it is found in greatest concentration. In *Trichosurus* and *Pseudocheirus* little material, other than that giving a positive P.A.S. reaction, is found between the membranes and uterine epithelium. In *Protemnodon*, however, a large amount of material, apparently consisting of degenerating cells that have broken free from the uterine epithelium, is found in addition to the P.A.S. positive material (Pl. 4, figs. 19, 20). The uterine epithelium differs from that of *Trichosurus* and *Pseudocheirus* in that each of its cells is, in its greater part, separated from its neighbour by a gap so that it forms a peg-like projection into the uterine lumen (Pl. 4, figs. 19, 20). Certain epithelial cells, with pale staining nuclei, are in the process of breaking free from the epithelium. Some of the cells free in the lumen have a faintly staining nucleus but most are enucleate and often fragmented or in process of disintegration. This cell detritus appears to be absorbed by the cells of the embryonic membranes.

The embryonic blood cells are separated from the maternal blood cells by a thin embryonic endothelium, a well developed layer of mesenchyme, the embryonic ectoderm, the uterine epithelium, maternal connective tissue and the endothelial wall. These last two layers are well developed—much better

so than in *Trichosurus* or *Pseudocheirus*. The sub-epithelial capillary layer, compared with that of these two species, is poorly represented and the entire uterine endometrium is relatively non-vascular. No shell membrane is found in any region on the surface of the membranes.

The embryonic membranes of the late embryo are essentially similar to those of the early embryo. In the mucosa however the gaps separating the peg-like epithelial cells are greater and the whole maternal epithelium appears depleted of cells. Since no mitoses occur in the epithelial cells of the pregnant uteri of either of these stages there exists no mechanism by which the cells contributing to the detritus in the lumen may be replaced. There is less of this cell detritus in the late embryo but this may be because breakdown and absorption of the free cells is proceeding at a faster rate due to the closer apposition of embryonic and maternal tissues.

Material giving a positive histochemical reaction for iron is located in the basal portions of the epithelial cells of some uterine glands in pregnant and non-pregnant uteri of the female carrying the late embryo (Pr2). No reaction was obtained in the embryonic membranes or in the embryo itself. The test was completely negative in all other species.

(d) *Setonix brachyurus*

The epithelium of the uterus containing a 19 day embryo (S8) consists of regularly placed cells with deeply staining spherical to ovoid nuclei. The sub-epithelial capillary layer is very poorly developed and blood vessels are few elsewhere in the mucosa. Leucocytes occur occasionally in the sub-epithelial capillaries and in other maternal blood vessels. P.A.S. positive material occurs in the gland cavities and sparingly on the inner surface of the maternal uterine epithelium. A slight reaction occurs within the embryonic membranes and this material gives positive histochemical tests for glycogen whereas the other P.A.S. positive material does not.

As development proceeds the cells of the uterine epithelium become successively distorted and flattened where they make contact with the embryonic membranes (Pl. 4, fig. 18). The epithelium, however, remains complete throughout pregnancy. The sub-epithelial capillary layer becomes better developed and the general vascularity of the uterus increases as pregnancy advances but at no time does it approach the condition found in *Pseudocheirus*. Leucocytes become more plentiful in the blood vessels and at 21 days (S9) appear in the glands and lumen of the uterus. In some cases at least, the leucocytes enter the uterine lumen by discharge from the gland mouths. They presumably contribute to the embryotrophe since they are seen in places in process of disintegration within the membranes (Pl. 4, fig. 21). As in *Pseudocheirus* there are projections of the uterine mucosa which contain maternal capillaries, but no glands, into the uterine lumen. These are but poorly developed however.

Embryonic and maternal blood streams are separated by the two endothelia, by embryonic mesenchyme and maternal connective tissue and by the extra-embryonic ectoderm and maternal uterine epithelium. At 25 days the connective tissue and endothelium on the inner sides of some of the maternal

capillaries, proximal to the membranes, is flattened and enucleate but in general the two blood streams are not brought into close proximity.

In later stages of pregnancy the P.A.S. positive material, both within the glands and uterine lumen, increases compared to the amount found in the 19 day embryo. Much of the material within the lumen enters from the mouths of the glands but some is formed within the lumen. At 25 days the greatest amount of P.A.S. positive material per unit area is found in the region of the chorion, less occurs in the region of the vascular omphalopleure, little in the region of the bilaminar omphalopleure. Further P.A.S. positive material, which is digested by saliva and which gives a positive reaction for glycogen, is found inside the cells of the various membranes and also within the uterine epithelial cells. The strongest reaction for glycogen is found in the cells of both layers of the chorion and in the adjacent uterine epithelium. In the ectoderm and endoderm cells of the vascular omphalopleure, and in the uterine epithelium in this region, a weaker reaction occurs. Droplets of glycogen also occur within the vessels of the vascular omphalopleure and those of the yolk-sac splanchnopleure. A little occurs in ectoderm and endoderm cells of the bilaminar omphalopleure and a trace occurs in the epithelial cells adjacent to this membrane. Extra-cellular glycogen undoubtedly occurs on the inner surface of the chorion within the extra-embryonic coelom, it perhaps also occurs in small quantity within the cavity of the yolk-sac in the region of the vascular omphalopleure.

The embryonic membranes are retained in the uterus at parturition as in several other marsupials (Amoroso, 1952). In a female killed 26 days after mating (S14) the embryo was found in the median vaginal culs-de-sac and embryonic membranes were seen in the sectioned uterus.

(e) *Potorous tridactylus*

In the only embryo studied the shell membrane is largely broken down in the region of the bilaminar omphalopleure although it is complete in the region of the vascular omphalopleure. The chorion is but poorly developed and consists of two layers of flattened cells (Pl. 4, fig. 22) except in a few regions where the ectoderm cells are large and in close contact with the uterine epithelium. The epithelial cells of the uterine lumen are produced into the same peg-like processes, projecting into the lumen, as occur in *Protemnodon*. The sub-epithelial capillary layer is poorly developed and blood vessels are not common deeper in the mucosa. A small amount of P.A.S. positive material occurs in the uterine glands. Leucocytes are found in the connective tissue separating the glands and blood vessels of the uterine mucosa but are not seen in the blood vessels themselves. These leucocytes presumably migrate through the connective tissue and uterine epithelium for they are commonly found within the uterine lumen. Here they serve an embryotrophic function since they are found in various stages of degeneration within the embryonic membranes.

DISCUSSION

In 1895 Hill showed that the marsupial *Isoodon obesulus* (=*Perameles obesula*) had a true allantoic placenta of a complex type and that the description

" aplacental " could no longer be applied to the marsupials as a group. Hill (1898) believed that *Perameles* was a primitive marsupial and that those marsupials which have only yolk-sac placentation were derived from ancestors with a complex syncytialised allantoic placenta like that of *Perameles*. Flynn (1922, 1923 b) although disagreeing with Hill about its method of formation, also believed that the placenta of *Perameles* represented the primitive condition for marsupials. Hill (1932, 1949), after a re-examination of his material, confirmed the conclusions of Flynn about the method of formation of the placenta of *Perameles* but now concluded it did not have the primitive significance he originally attached to it. He suggested instead that the most primitive placental type amongst marsupials was a combined allantoic and yolk-sac placenta of the simple apposed type and this gave rise to both the allantoic placenta of *Perameles* and to the purely yolk-sac placentae of other marsupials. It is today generally accepted that marsupials are descended from ancestors with allantoic placentation but Pearson (1947, 1949) has expressed the view that the yolk-sac placenta of most marsupials represents the primitive condition. The results of this investigation, and a study of the published descriptions of the embryonic membranes of other marsupials, suggest that Pearson's views are more acceptable than those of either Hill or Flynn.

The evolution of viviparity in mammals was presumably accompanied by a reduction in the shell, albumen and egg-yolk and a progressive increase in the tolerance of the uterus to the contained embryo. Since the yolk-sac is the first of the amniote embryonic membranes to become vascularised it is reasonable to assume that this was the first of the membranes to form a vascular placental structure—a function which it retains in the early uterine stages of living viviparous amniotes. In *Didelphis*, a generalised marsupial and a representative of the group considered ancestral to other marsupials (Simpson, 1945), the allantois is small and does not take part in placental formation but it is not necessarily secondarily reduced. During the short (13·5 day) gestation period the allantois does not have time to reach a larger size. In *Trichosurus*, which has a slightly longer gestation period than *Didelphis*, there is a greater area of vascular yolk-sac placenta and the allantois is slightly larger. The allantois remains enclosed by folds of yolk-sac splanchnopleure until the close of pregnancy. *Trichosurus* fulfils many of the requirements of a hypothetical ancestor of the macropod marsupials in which group the gestation periods are longer and there is an increased development of the embryonic membranes. The allantois of *Setonix*, *Protemnodon* and other macropods, however, becomes completely enshrouded by folds of the yolk-sac splanchnopleure and, although large, cannot fuse with the chorion. This line of placental evolution thus appears to have culminated in an arrangement of embryonic membranes precluding the possibility of evolution of allantoic placentation.

In *Pseudocheirus*, where the yolk-sac placenta forms a more intimate association with the uterine wall than in *Trichosurus*, the allantois reaches almost to the chorion and is only incompletely shrouded by the yolk-sac splanchnopleure. The condition in *Phascolarctos*, where the allantois reaches

the chorion (Semon, 1894), is thus foreshadowed by the condition in *Pseudocheirus* from which genus, according to Bensley (1903), *Phascolarctos* is derived. Jones (1924) also believed *Phascolarctos* was most closely related to *Pseudocheirus*. *Phascolarctos* is certainly one of the most specialized diprotodont marsupials so it is unlikely that it has retained the primitive type of marsupial placenta as Hill (1949) suggested.

Flynn (1922), when describing the maternal syncytium of *Perameles*, stated that a similar syncytial formation occurred in the pregnant uterus of *Pseudocheirus convolutor* (*P. cooki*). He interpreted this as evidence that *Pseudocheirus* was derived from an ancestor with a complex allantoic placenta like that of *Perameles*. My preparations of pregnant uteri of *Pseudocheirus peregrinus* do not, at any stage, show any resemblance to the syncytial formation in the pregnant uterus of *Perameles* either as described by Hill (1898, 1901), or Flynn (1923 b), or as seen in my own preparations of pregnant uteri of *Perameles nasuta*. Flynn also suggested that the syncytial structure of the uterus of the pregnant *Dasyurus* (Hill, 1900) was evidence that this marsupial was descended from an ancestor with allantoic placentation. It is at least equally likely that the condition in *Dasyurus* could represent the precursor of an allantoic placenta rather than a stage in its degeneration. The placental structures of only a fraction of the total marsupial species have so far been studied and allantoic placentation may have evolved in two or more separate lines.

Hartman (1952) stated that, in *Didelphis*, " transition from delicate vesicle smaller than a pinhead to newborn opossum requires only 6 days ". In *Trichosurus* and *Setonix* growth and differentiation of the embryo are also rapid in the closing stages of pregnancy. The embryonic vesicle of *Trichosurus* 12·5 days after mating measures 7·5 mm. diameter, the embryonic area is 3·5 mm. long and mesoderm is hardly extended beyond its limits. Two to three days later definite body form is established and organogenesis is well advanced. In about three more days the young is born.

In *Didelphis* (Hartman, 1923), *Dasyurus* (Hill & O'Donohgue, 1913) and *Trichosurus* (G. B. Sharman & Phyllis E. Pilton, unpublished) the post-ovulatory uterine changes, apparently influenced by the secretions of the corpus luteum, are of the same degree and duration in pregnant as in non-pregnant females. Parturition, in these species, is coincident with the beginning of degenerative changes in the corpora lutea. In *Setonix* the corpus luteum has a longer period of functional activity than in the above species and the young is retained in the uterus after the end of the luteal phase. The period of active secretion of the corpus luteum during pregnancy is not, however, longer than in the oestrous cycle and oestrus occurs, after parturition, at the time it would be expected had pregnancy not taken place (Sharman, 1955 b). There is thus no evidence for the secretion of a placental luteotrophic principle which prolongs the period of active secretion of the corpora lutea of any of these marsupials during pregnancy. Amoroso (1955) suggested that the secretion of such a principle was the initial step, in the evolution of viviparity, which enabled the embryonic mammal to be retained in the uterus beyond the limits of the oestrous cycle. In this context the marsupial *Potorous*

is of particular interest for, in this species, pregnancy is longer than in most marsupials but the oestrous cycle is also long and, as in other marsupials, exceeds the gestation period. In the above series of marsupials those which, by all the currently accepted criteria, are regarded as advanced have the longest gestation periods. This appears to conflict with Jones' (1923) conclusion that evolution in marsupials had been accompanied by abbreviation of the gestation period (see Sharman, 1959).

In the marsupials studied in this investigation the bilaminar omphalopleure is undoubtedly an important organ of embryonic nutrition in early pregnancy but in later stages its functions are increasingly taken over by the vascular omphalopleure. Histotrophic materials are ingested by way of the vascular omphalopleure in *Protemnodon* and *Setonix* and in all species the periodic acid-Schiff and other histochemical reactions are strongest in the region of the vascular omphalopleure in late stages of pregnancy. These observations are of interest in view of Hill's (1900) conclusions that the vascular omphalopleure of *Dasyurus* serves only a respiratory function whereas embryotrophic nutrition is carried out solely by the bilaminar omphalopleure. In *Dasyurus* the gestation period is shorter than that of any of the species studied in this investigation so it is to be presumed that increase in the gestation period of marsupials has been accompanied by progressive development of the vascular omphalopleure as an organ of embryotrophic nutrition. The vascular omphalopleure of *Bettongia*, which has a very long gestation period by marsupial standards, is also an important organ of embryotrophic absorption (Flynn, 1930).

The absence of stainable iron in the embryonic membranes of all the marsupials studied is of some interest. In eutherian mammals iron enters the embryo by placental transfer and it may be readily demonstrated, by histochemical methods, in the embryonic membranes of various species (Amoroso, 1952 ; Huggett & Hammond, 1952). Since there is a shortage of iron in the milk of eutherians there is a decline in blood haemoglobin and in storage iron in the young animal during the suckling period. In the marsupial *Setonix brachyurus* it has been shown by Kaldor (cited by Barker & Barker, 1959) that the milk has an unusually high concentration of iron and that the blood haemoglobin concentration of the young rises, from an initial low level, throughout pouch life.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1—Reproductive system from pregnant female of *Trichosurus vulpecula* (T5, 16·5 days from mating). cl, corpus luteum ; lv, lateral vagina ; pu, pregnant uterus ; u, urethra ; ugs, urogenital sinus.

" 2—Dissected pregnant uterus of *Setonix brachyurus* (S9, 21 days). Photo taken looking into the anterior end of the uterus. ay, allantois beneath yolk-sac splanchnopleure ; bom, bilaminar omphalopleure ; st, sinus terminalis ; um, uterine mucosa ; va, vitelline artery ; vom, vascular omphalopleure ; vv, vitelline vein ; ys, yolk-sac splanchnopleure.

Fig. 3—Dissected pregnant uterus of *Setonix brachyurus* (S12, 25 days). Lettering as in Fig. 2.
" 4—Dissected pregnant uterus of *Trichosurus vulpecula* (T3, about 15 days). eay, embryo beneath yolk-sac splanchnopleure and amnion; ep, embryo beneath pro-amnion; un, uterine neck. Other lettering as in Fig. 2.
" 5—Dissected left pregnant uterus of *Pseudocheirus peregrinus* (P2). Note the large size of the allantois invested by the yolk-sac splanchnopleure (ay).

Plate 2

Fig. 6—L.S. right pregnant uterus of *Pseudocheirus peregrinus* (P1). bom, bilaminar omphalopleure; ul, uterine lumen; um, uterine mucosa; vom, vascular omphalopleure; yc, yolk-sac cavity.
" 7—Newborn young of *Trichosurus vulpecula* (about 12 hours old) in mother's pouch. t, teat.
" 8—L.S. pregnant uterus of *Potorous tridactylus*. ac, allantoic cavity; am, amnion; c, chorion; con, chorio-amniotic connection; ec, extra-embryonic coelom; pao, pro-amnion, applied to epithelium of embryo; st, sinus terminalis; um, uterine mucosa; vom, vascular omphalopleure; yc, yolk-sac cavity; ys, yolk-sac splanchnopleure.
" 9—L. S. pregnant uterus of *Trichosurus vulpecula* (T5, 16·5 days). amy, fused amnion and yolk-sac splanchnopleure. Other lettering as in Fig. 8.

Plate 3

In all figures the uterine lumen appears above the embryonic membranes, the uterine mucosa below the membranes.

Fig. 10—Bilaminar omphalopleure of *Trichosurus vulpecula* (T4). Ectoderm and endoderm of bilaminar omphalopleure consists of flattened cells with elongated nuclei. bom, bilaminar omphalopleure; pas, periodic acid-Schiff positive material; sm, shell membrane.
" 11—Bilaminar omphalopleure of late embryo of *Trichosurus vulpecula* (T5). Compare with Fig. 10. mbv, maternal blood vessel.
" 12—The cell layers separating the embryonic and maternal blood streams in the pregnant uterus of *Trichosurus vulpecula* (T5, 16·5 days). Photograph shows sinus terminalis (ebv) with bilaminar omphalopleure at right. The nucleated embryonic red blood corpuscles are separated from the maternal corpuscles in the capillaries (msc) by six layers of cells. These are the embryonic endothelium (ec), embryonic mesenchyme (em), embryonic ectoderm (ecc), the uterine lumen epithelium (ule), maternal connective tissue (not obvious in figure) and the maternal endothelium (me). The membranes have separated from the uterine mucosa during preparation.
" 13—Vascular omphalopleure of *Trichosurus vulpecula* (T5), showing embryonic capillaries (eca) and maternal subepithelial capillaries (msc). ug, uterine gland; yc, yolk-sac cavity.
" 14—Vascular omphalopleure of *Pseudocheirus peregrinus* (P2). In the area selected for illustration the membrane has pulled away from the uterine mucosa during preparation. Embryonic capillaries (eca) and maternal sub-epithelial capillaries (msc) are brought close to the surfaces by displacement of nuclei from overlying layers. The uterine lumen epithelium and a layer of maternal connective tissue, both thin and enucleate, may be seen just below and to the right of the maternal subepithelial capillary.
" 15—Vascular omphalopleure of *Pseudocheirus peregrinus* (P2) showing close apposition of embryonic capillaries (eca) and maternal subepithelial capillaries (msc).

Plate 4

Fig. 16—Vascular omphalopleure of *Pseudocheirus peregrinus* (P2) showing clusters of embryonic mesoderm cells (emc) and close apposition of foetal and maternal tissues. msc, maternal sub-epithelial capillary.
" 17—Sinus terminalis (st), vascular omphalopleure (vom) and bilaminar omphalopleure (bom) with overlying shell membrane (sm) in an early embryo of *Setonix brachyurus* (S8, 19 days).
" 18—Vascular omphalopleure (vom), of late embryo of *Setonix brachyurus* (S12, 25 days). Subepithelial capillaries (msc) and blood vessels are few in the uterine mucosa. ule, uterine lumen epithelium.

Fig. 19—Bilaminar omphalopleure and uterine lumen epithelium (ule) separated by cell detritus (cd) in *Protemnodon rufogrisea* (Pr1).

" 20—Vascular omphalopleure of *Protemnodon rufogrisea* (Pr1). Maternal subepithelial capillaries (msc) are few in the uterine mucosa. cd, cell detritus; ule, uterine lumen epithelium.

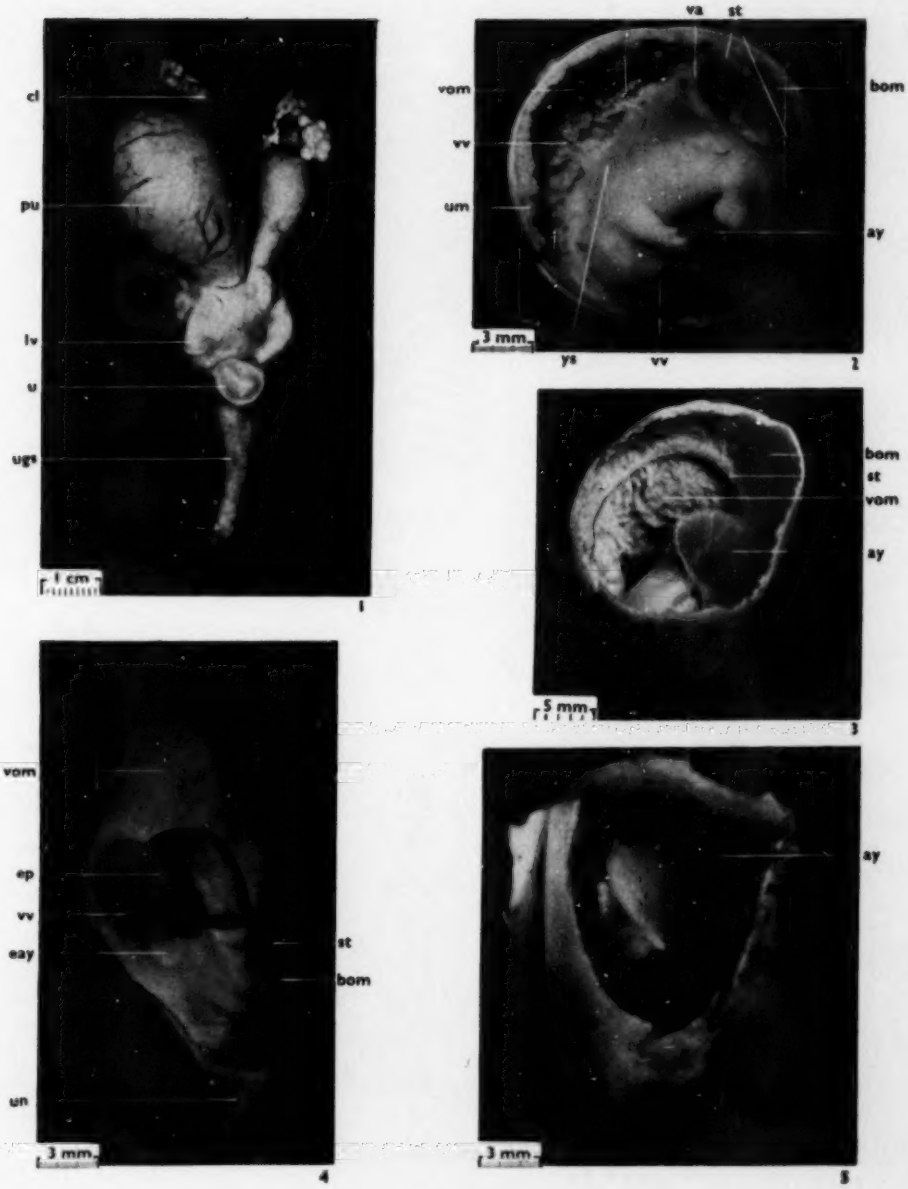
" 21—Chorion of *Setonix brachyurus* (S12). ld, degenerating leucocytes; ule, uterine lumen epithelium.

" 22—Chorion (c) and uterine lumen epithelium (ule) of *Potorous tridactylus*.

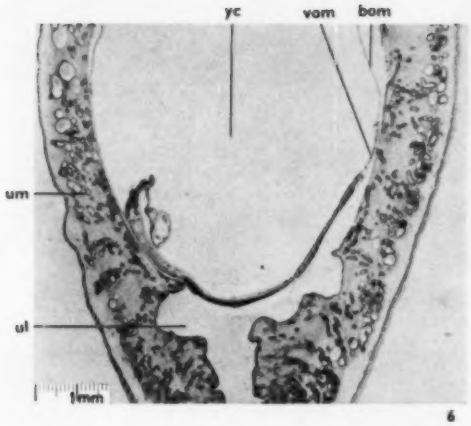
APPENDIX

General localities, ages (time from mating) and stages of development of embryos studied.

No.	Date	Age (days)	Stage	Position
		Trichosurus vulpecula Adelaide, South Australia.		
T1	1–iii–57	unknown	4 cell	left uterus
T2	23–viii–57	12·5	7·5 mm. dia. vesicle, embryonic area 3·5 mm. long	right uterus
T3	29–iii–57	ca.15	embryo not measured	right uterus
T4	26–vii–57	ca.15	embryo 6·5 mm. G.L.	left uterus
T5	5–vii–58	16·5	embryo 11·0 mm. G.L.	right uterus
		Pseudocheirus peregrinus Delamere, South Australia.		
P1	29–v–58	unknown	embryo 5·4 mm. G.L.	right uterus
			embryo not measured	left uterus
P2	29–v–58	unknown	embryo 14·1 mm. G.L.	left uterus
			embryo not measured	right uterus
		Protemnodon rufogrisea Temma, Tasmania.		
Pr1	20–ii–53	unknown	embryo 7·0 mm. G.L.	uterus
Pr2	20–ii–53	unknown	embryo 10·3 mm. G.L.	uterus
		Setonix brachyurus Rottnest Is., Western Australia.		
S1	11–vi–53	2	egg, pronuclei not fused	right tube
S2	20–vii–53	5	0·3 mm. unilaminar blastocyst	left uterus
S3	26–ii–53	unknown	12 mm. vesicle	right uterus
S4	26–ii–53	unknown	embryo 6·6 mm. G.L.	right uterus
S5	22–vi–55	17	embryo 4·0 mm. G.L.	left uterus
S6	14–viii–52	18	embryo 4·8 mm. G.L.	right uterus
S7	8–vi–56	19	embryo not measured	left uterus
S8	24–vi–55	19	embryo 4·8 mm. G.L.	left uterus
S9	10–vi–56	21	embryo 8·6 mm. G.L.	right uterus
S10	28–ii–53	unknown	embryo 9·5 mm. G.L.	left uterus
S11	28–vi–55	23	embryo 11·0 mm. G.L.	left uterus
S12	30–vi–55	25	embryo 11·3 mm. G.L.	left uterus
S13	26–ii–53	unknown	embryo not measured	right uterus
S14	18–vi–53	26	degenerating embryonic membranes	right uterus
			embryo 14·0 mm. G.L.	birth canal
		Potorous tridactylus Dunorlan, Tasmania.		
Pt1	17–i–57	unknown	embryo 5·2 mm. G.L.	right uterus



Embryonic membranes of marsupials.



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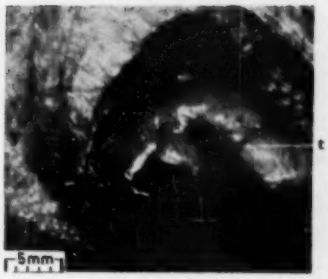

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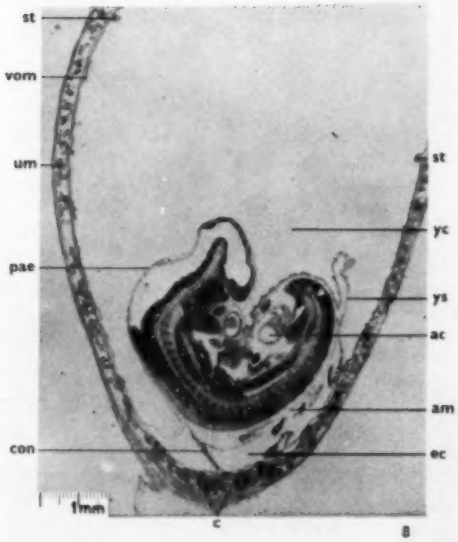

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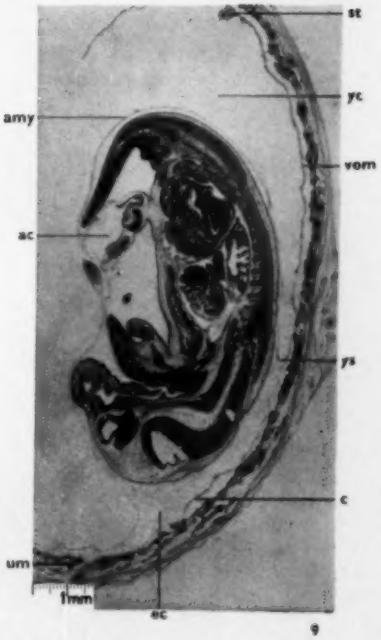

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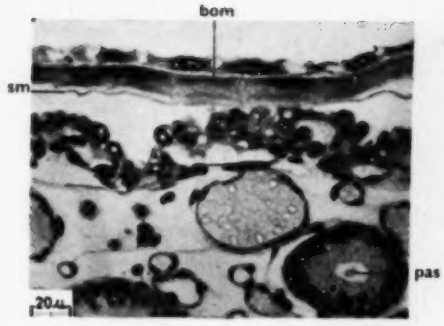

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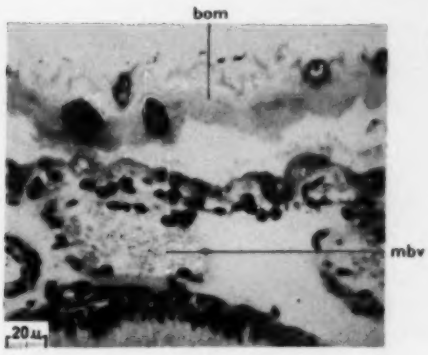

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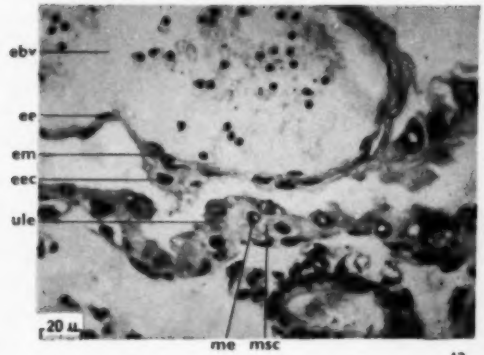

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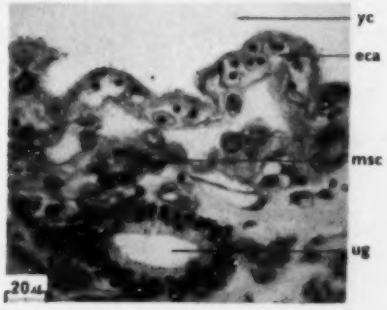

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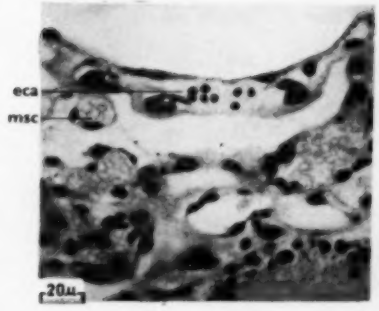

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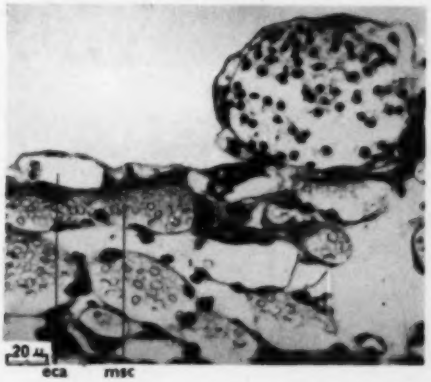

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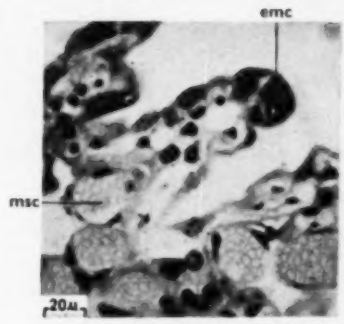
Embryonic membranes of marsupials.



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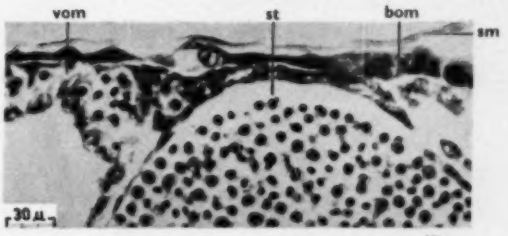

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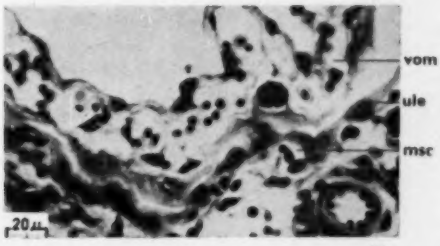

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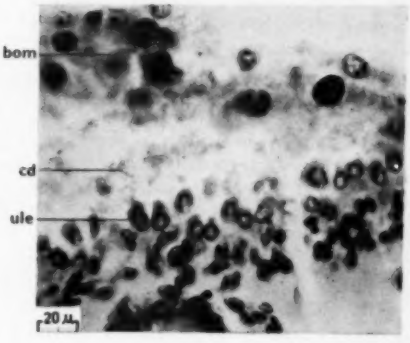

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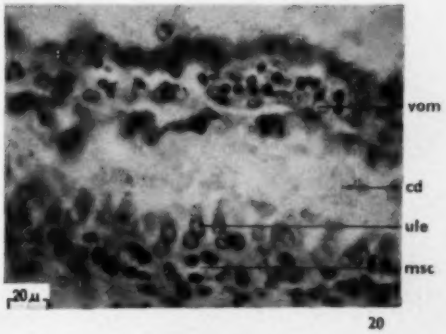

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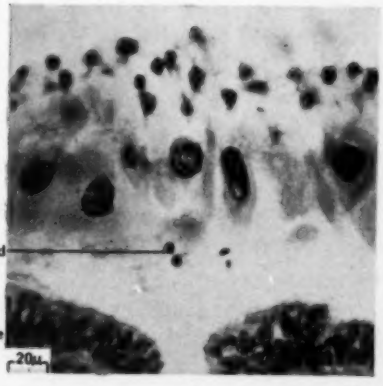

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Embryonic membranes of marsupials.

THE LIFE HISTORY AND ECOLOGY OF THE LITTORAL CENTIPEDE *STRIGAMIA* (=*SCOLIOPLANES*) *MARITIMA* (LEACH)*

BY

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[Accepted 14th February, 1961]

(With 14 figures in the text)

The work here reported is of an investigation into the ecology and life history of a shore dwelling geophilomorph centipede *Strigamia maritima* (Leach). A method of determining the age of individuals and hence the life history, is described. By analysis of monthly samples it is shown that for the purposes of insemination, egg laying and moulting, individuals migrate from their feeding grounds, which are frequently covered by the tide, to a shingle bank where more terrestrial conditions prevail. The egg-laying period is more restricted than in terrestrial species and takes place in May and June when spring tides are at their smallest amplitude and there is consequently least danger of the eggs being immersed. When animals in the feeding zone are covered by the tide they migrate out of this zone up into the shingle bank. It is concluded that the species is able to maintain itself in the intertidal zone only by virtue of the fact that non-saline conditions prevail at the top of the beach for much of the year. Information on the prey, predators and parasites of *S. maritima* is given.

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INTRODUCTION

Of the four species of entirely littoral geophilomorph centipedes known to occur in the British Isles, *Strigamia* (=*Scolioplanes*) *maritima* (Leach) is the most common (Blower, 1955 a). It has been reported from a number of

* This work formed part of a thesis approved for the award of the Degree of Ph.D. by the University of London.

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localities throughout the British Isles (Cloudsley-Thompson, 1948, 1951). The species has normally been referred to the genus *Scolioplanes* by British workers, but recently Crabill (1953) has shown that the generic name *Strigamia* has preference.

The species occurs in both rock crevices (Glynne-Williams & Hobart 1952, Morton 1954, Turk & Turk 1958), and shingle banks (Pocock 1906, Newell 1954, Eason 1957) ; it does not appear to be present in muddy or sandy shores. The animal frequently occurs in large numbers ; thus Bagnall (1917) took several hundred examples on one occasion, while Pocock (1900) speaks of " swarms " of the species, and dense populations were frequently found during the present investigation. In this respect the species is unusual, since geophilomorphs from other habitats are generally not taken in large numbers. The high density which obtains in populations of *S. maritima* makes this species particularly suitable for studies on life history.

THE HABITAT

The population studied inhabited a shingle bank on the east side of the mouth of the River Cuckmere which enters the English Channel some two and a half miles east of Seaford, Sussex (National Grid reference 50/518977). The shingle bank, which runs east-west is backed mostly either by *Obiene* salt marsh,

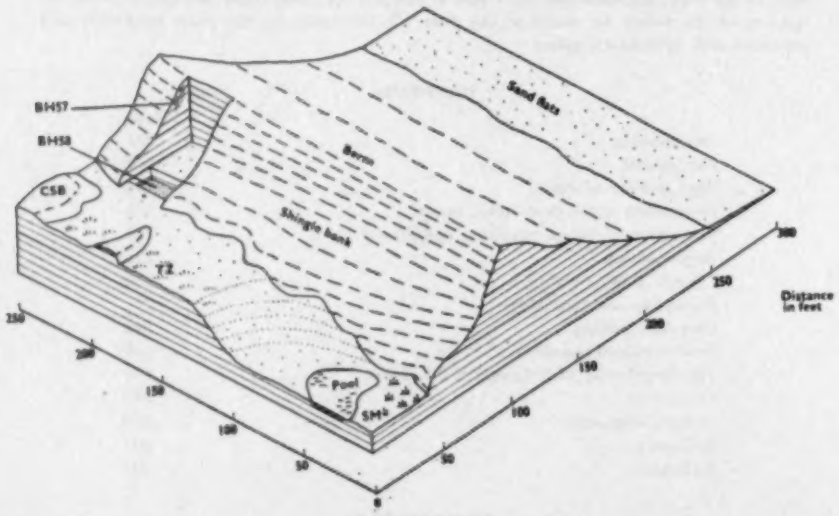

Fig. 1—A three dimensional reconstruction of part of the beach at Cuckmere Haven contoured at one foot intervals.

BH57, 1957 brooding habitat ; BH58, 1958 brooding habitat ; CSB, consolidated shingle bank ; SM, salt marsh ; TZ, zone of transition to meadow.

or by consolidated shingle, both of which grade into meadow behind (Fig. 1). On its seaward side, which is continually being eroded and rebuilt by the tide, it slopes down to sand flats at about mid tide level. The fauna of the bank

was usually found to be most dense in the decaying organic matter below the storm line which is situated several feet landwards of the berm. Elsewhere in the bank the fauna was usually sparse, though drift lower down the shore sometimes supported dense populations of the talitrid *Orchestia gammarella* (Pallas) and the kelp fly *Orygma luctuosum* Meigen.

Although *Strigamia* was almost completely absent from the salt marsh proper, large numbers of individuals were frequently found at its edge in a narrow belt a few feet wide. Here the mud of the marsh is covered by a layer of shingle beneath which exists a large population of the isopod crustacean *Sphaeroma* sp. The centipede was frequently seen to be feeding on this animal.

Towards the eastern end of the beach the main shingle bank is backed by a low consolidated shingle bank which probably represents the remains of an earlier shingle spit. It supports a sparse flora of species of *Rumex*, *Artemisia*, *Sedum* and of the seakale *Crambe maritima* L. The shingle here is much compacted, containing a fair amount of sand and a poor loam round the plants. The fauna is largely terrestrial in character, the most typical animals being woodlice, ants, and the earwig *Forficula auricularia* L. *Strigamia* was normally absent from this region, but in November 1957 the species was common beneath the inch or two of loose shingle which covered the bank. By December, however, the centipede had deserted this habitat and was not found there in such numbers during the rest of the investigation. The reason for this migration is unknown.

In the summer of 1957 females with eggs and young were found in a six inch deep layer of sand and fine shingle about twenty-five feet landwards of the berm of the main shingle bank at a depth of about a foot. The number of such "brooding" females was very high, broods being sometimes only a few millimetres apart. Unfortunately this region of the beach was extensively bulldozed during the following winter and the "1957" brooding habit was destroyed.

In 1958 a search was made for other brooding sites, and one was found at the inner edge of the shingle bank where it meets the consolidated shingle. Brooding females were found here at a depth of about a foot between an inch thick layer of sandy mud and the underlying sand (Fig. 1). In 1959 brooding females were again common here. This region was termed the 1958 brooding habitat.

EGGS AND LARVAL STAGES

At Cuckmere, female *S. maritima* lay their eggs at the end of May or the beginning of June in small brood cavities hollowed out of the sand in the brooding habitat. The eggs are spherical and measure from 0·9 to 1·25 mm. in diameter. The shell is of a yellowish khaki colour and somewhat elastic. It is, however, easily ruptured.

The eggs hatch towards the end of June, and the larval stages closely resemble those described by Verhoeff (1902–25) in *Mecistocephalus carniolensis* C. L. Koch. His terminology is here adopted. Hatching is a very gradual process in *Strigamia*, the egg shell splits equatorially, and as the embryo, which

is bent into a horseshoe shape, gradually elongates and uncurls during development, it pushes the two halves of the shell apart. In *S. maritima* hatching begins at what Verhoeff terms the last embryonic stage, in which the limbs and mouthparts are represented by simple buds, and the body, covered with cuticle, is inflated anteriorly since it still contains a large quantity of yolk.

Eggs which are about to hatch appear pinkish owing to the pinky-violet colour of the contained embryos. The pigment occurs in the gut cells of the larvae, the rest of the body being whitish and translucent. The last embryonic stage moults to the "peripatoid" stage in which the trunk is of uniform diameter, and the limbs better developed. These two stages are not distinguished in Table 1, both being entered under "peripatoid". The peripatoid stage is immobile, but the "foetus" stage which succeeds it is capable of making writhing movements; it has fully jointed limbs and antennae, and clearly recognisable mouthparts. This stage is dorso-ventrally flattened and averages 7 mm. in length. The first adolescens stage is the last to be brooded by the female and closely resembles the adult in form. In it the gut has darkened, losing its pinky-violet colouration, and the cuticle has hardened. The

Table 1—State of development of clutches of *Strigamia* eggs and young found at Cuckmere Haven from 1957 to 1959.

Date	Eggs unlaid	Eggs laid	Broods at peripatoid stage	Broods at foetus stage	Broods at Adolescens I stage
1957					
June 2nd	14	1			
June 16th	1	16			
July 1st	—	1	4	2	
July 15th	—	—	—	4	
August 2nd	—	—	—	—	3
1958					
May 21st	12	1			
June 9th	—	8			
June 24th	—	3	1		
July 7th	—	—	—	8	
July 21st	—	—	—	8	4
July 28th	—	—	—	2	6
August 6th	—	—	—	—	11
1959					
June 30th	—	1	5		
July 7th	—	—	2	4	

adolescens I is more elongated than the peripatoid stage, is oval in cross section and about 10 mm. long. In its behaviour, however, the newly moulted adolescens I resembles the foetus since it can only perform squirming movements. Older specimens can crawl, but tend to crawl in circles. Specimens which are older still crawl in straight lines. Insufficient data were collected to enable any accurate estimate of the time for which the female brooded the

adolescens I to be made, but the stage is undoubtedly brooded until such time as the young have developed the full adult behaviour pattern.

Examination of Table 1 shows that during the three years of the investigation the period during which the females were found brooding eggs was very limited ; the earliest record was on the 21st May, the latest on 1st July. This contrasts with the situation in other geophilomorphs where females are found with eggs over a much longer period. In the North American species *Geophilus rubens* Say for example, Johnson (1952) found eggs from 14th June to 10th August and in Finland Palmen and Rantala (1954) found female *Pachymerium ferrugineum* C. L. Koch brooding eggs from 22nd May to 2nd September. For the two British terrestrial species *Geophilus carpophagus* Leach and *Brachygeophilus truncorum* (Bergsoe and Meinert) Vaitilingham (1960) found the egg laying period to last from the beginning of May almost to the end of August.

A possible explanation for the restriction of the brooding season in *S. maritima* may be the need to protect the eggs and delicate larval stages from immersion during the gales and higher tides occuring in the autumn. It may be significant that the eggs and larvae are found at the time of the lowest ranging spring tides of the year, and are laid at sites well out of reach of the summer tides. Eggs collected in 1958 shrank rapidly in sea water, but the only three collected in 1959 showed only a slight initial shrinkage which was followed by recovery. Examination showed these eggs to be in a fairly advanced state of development and it may be that they possess some form of regulating mechanism at this stage. Although peripatoid stage *Strigamia* larvae shrank rapidly and soon died in unsaturated air in the laboratory, they appeared to be unharmed after having floated on sea water for four hours.

DISTINCTION OF THE POST-LARVAL STAGES

Since the Geophilomorpha are epimorphic, hatching with their full complement of legs, leg number cannot be used in differentiating developmental stages as in the Diplopoda and Lithobiomorpha.

Verhoeff (1902–25) distinguished three pre-adult "adolescens" stages in the geophilomorph *Mecistocephalus carniolensis* and found that the most useful characters for doing this were the number of pores on the inflated coxae of the last pair of legs and the formation and chaetotaxy of the genital zone. Other characters which he found increased with age were the number of hairs on the clypeus, the antennae, the presternites, and "on various other regions of the body ".

The same worker described a first adolescens stage from *Geophilus carpophagus* together with a further stage which he designated "adolescens x" and which he suggested was a third or even a fourth adolescens stage. In this species the stages were distinguished by the number of coxal glands and by the structure of the carpophagous pits which are present on the anterior borders of the sternites.

Demange (1943) described small chaetae, "microchètes" from segments five, nine and thirteen of the antennae of *Hydroschendyla submarina* (Grube), which varied in number with the developmental stage of the species, and which he considered a specific or generic character.

Samples of the *Strigamia* population at Cuckmere were examined with respect to the above characters in an attempt to find some way of ageing individual specimens.

The species lacks carpophagous pits, but contrary to expectation microchètes are present on the antennae as in *Hydroschendyla* ; they are not, however, any more abundant in mature than in immature animals, averaging four on segment five, eleven on segment nine and eight on segment thirteen in all post-larval stages. Similarly the number of hairs on the clypeus does not change with age. Both the number of whorls of hairs on the antennae and on the last pairs of legs in females increase with age, the former from three or four in the smallest individuals to four or five in mature animals, the latter from one in the smallest to three or four whorls in mature animals, but these differences are not large enough to enable the characters to be used in distinguishing consecutive developmental stages. There is a marked increase, however, in the number of hairs on the pre-sternites, the smallest individuals having three to four and the mature forms ten to fifteen. Unfortunately, the difficulty experienced in counting these hairs, renders the character unsuitable for ageing large numbers of animals. Although there are marked differences in the structure of the genital zone in the younger stages, the structure of this region is of no value in separating the various age groups of mature and near mature specimens.

The remaining characters, namely the hairiness of the genital region in males, and the number of coxal glands on the last pair of legs in both sexes, were used in distinguishing developmental stages, and are discussed further below.

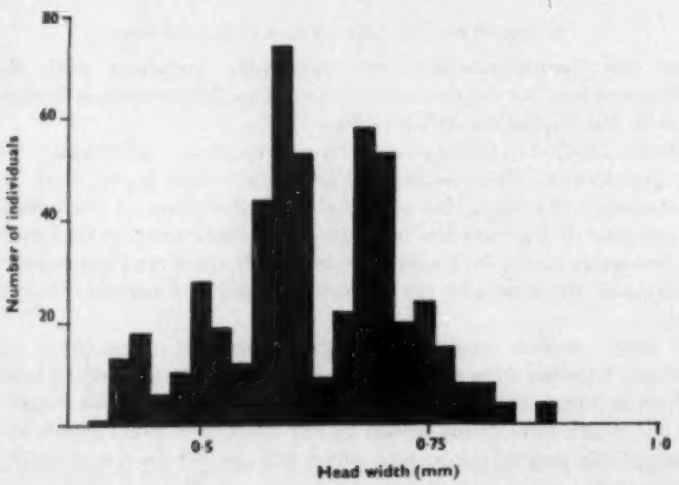

Fig. 2—Distribution of head widths in male *S. maritima*.

In addition to the above-mentioned characters, the width of the head capsule was used as a measure of the stage of development. The other measurements obtainable from heavily chitinised structures in *Strigamia*, namely the

head length and the length of the prehensors (poison claws) were affected by the tilt of the head in the narcotised animal and were therefore unreliable.

The distribution of head widths in a population sample of both sexes collected throughout the year is shown in Fig. 2 for males, and in Fig. 3 for females. Both histograms are at least quadrimodal suggesting that there is a minimum of four post-larval developmental stages in each sex. An analysis

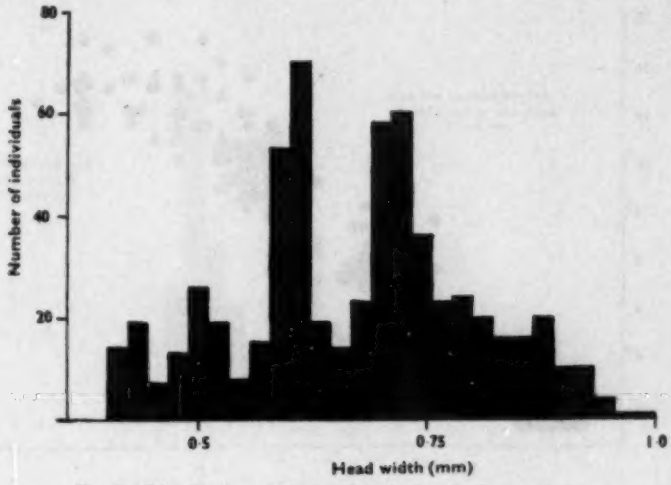

Fig. 3—Distribution of head widths in female *S. maritima*.

of these data using Hazen's probability paper (Harding, 1949) suggests that there are at least five populations or developmental stages involved for females, since there are four definite points of inflection in the curve obtained when a plot of these measurements expressed as a cumulative percentage is made. In males a similar plot shows only three definite points of inflection and thus the sample contains a minimum of four populations.

In order to determine rates of increase in weight it is necessary to be able to assign each individual to a particular instar. The head width data are not precise enough to enable this to be done. However, the number of glands opening on the inflated coxae of the last pair of legs increases with each instar, and if the average for both coxae is plotted against head width, a division of the population into four groups is obtained in each sex (Fig. 4 and 5). The fourth and largest group obtained for the female *Strigamia* contains the two instars whose existence was indicated by the graphical analysis on probability paper. Observations on moulting animals in the laboratory confirmed that this was so, for whereas an animal with an average number of coxal glands of 13·5 increased it to 15 on moulting, a second specimen with an average coxal gland number of 15·5 did not increase this. The structure of the seminal receptacles which is dealt with below, further supports this hypothesis.

In males a plot of the number of hairs on what Brolemann (1930) terms the first genital sternite separates the third and fourth groups more clearly and

reveals the presence of a fifth (Fig. 6). When the number of hairs on the first genital sternite is plotted against head width a fairly good separation of these three groups is obtained (Fig. 7). Unfortunately the number of hairs on the first genital sternite is of little value in separating these stages in the female.

Thus it is possible to obtain a fair separation of five post larval stages in female *Strigamia maritima* by using head width with the average number of

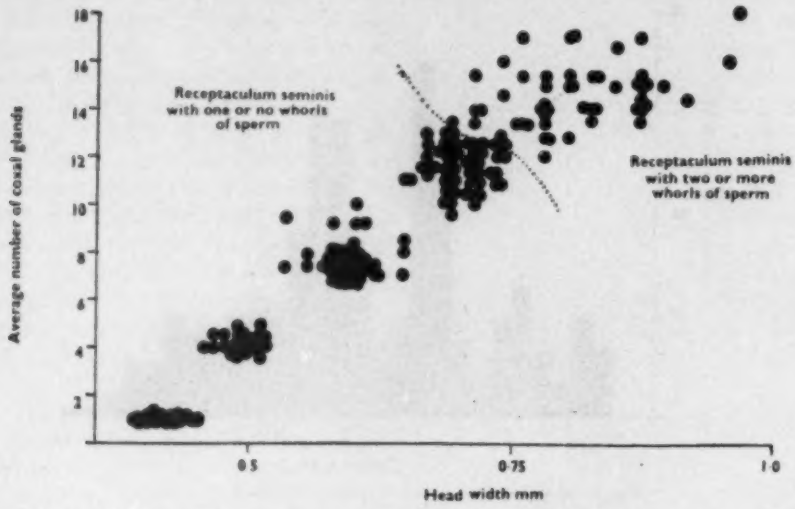

Fig. 4—A plot of head width against the average number of coxal glands in female *S. maritima*.

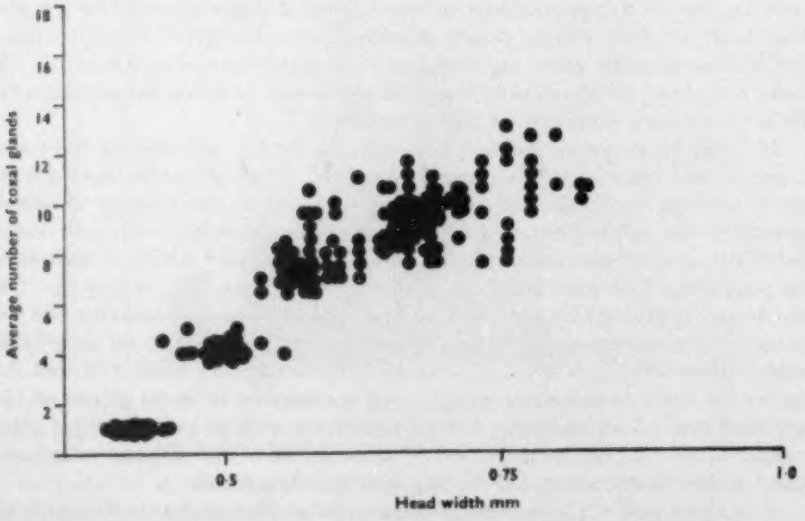

Fig. 5—A plot of head width against the average number of coxal glands in male *S. maritima*.

coxal glands, and a good separation in males by using head width and coxal gland number for the first two stages, and head width and the number of hairs on the genital sternite to differentiate a further three stages.

Of these five post-larval stages the fourth and fifth are mature, by which is meant they contain developing gametes which will be deposited during the next breeding season. They will be termed the maturus junior and maturus

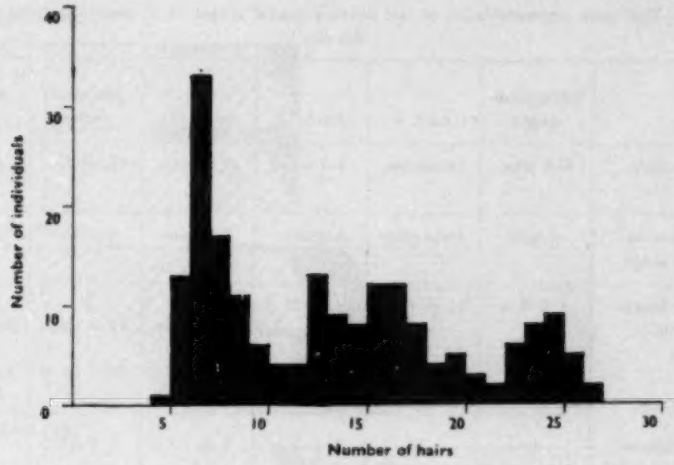

Fig. 6—Distribution of the number of hairs on the first genital sternite in male *S. maritima*. NB. The sample comprises adolescens III and maturus individuals only.

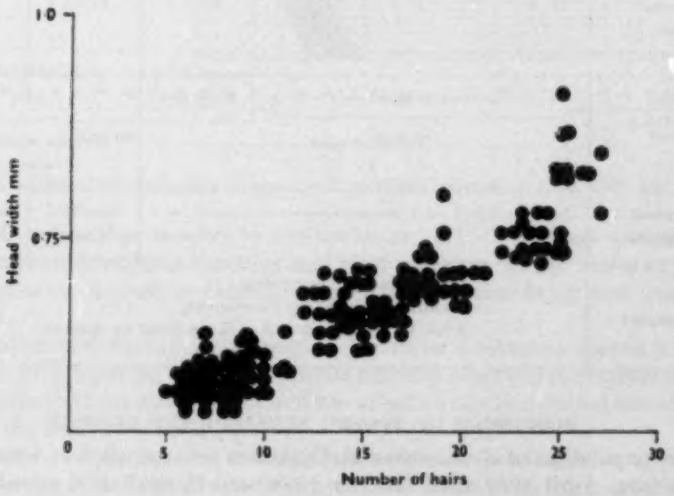

Fig. 7—A plot of the head width against the number of hairs on the first genital sternite in male *S. maritima*. NB the sample comprises adolescens III and maturus individuals only.

reveals the presence of a fifth (Fig. 6). When the number of hairs on the first genital sternite is plotted against head width a fairly good separation of these three groups is obtained (Fig. 7). Unfortunately the number of hairs on the first genital sternite is of little value in separating these stages in the female.

Thus it is possible to obtain a fair separation of five post larval stages in female *Strigamia maritima* by using head width with the average number of

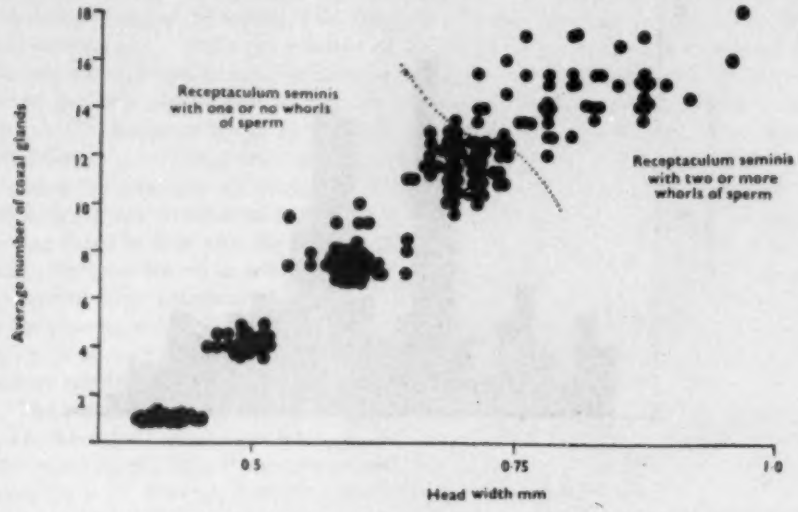
Receptaculum seminis with one or no whorls of sperm
Receptaculum seminis with two or more whorls of sperm
Average number of coxal glands
Head width mm
0·5
0·75
1·0

Fig. 4—A plot of head width against the average number of coxal glands in female *S. maritima*.

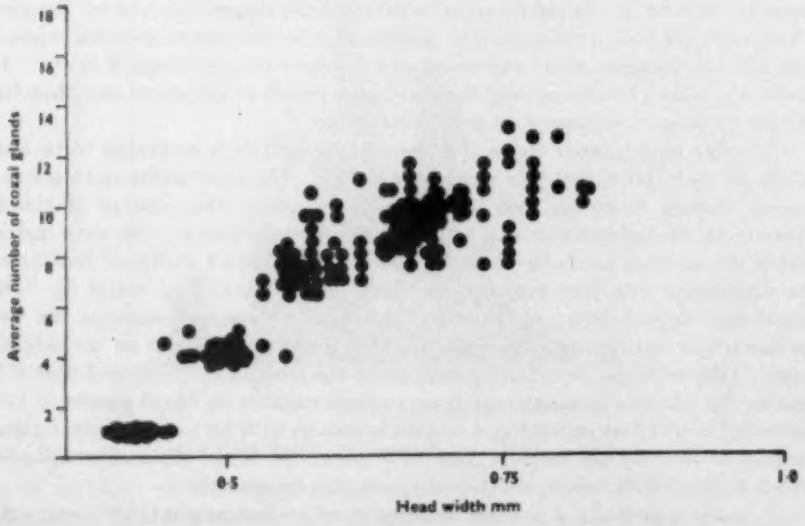

Fig. 5—A plot of head width against the average number of coxal glands in male *S. maritima*.

coxal glands, and a good separation in males by using head width and coxal gland number for the first two stages, and head width and the number of hairs on the genital sternite to differentiate a further three stages.

Of these five post-larval stages the fourth and fifth are mature, by which is meant they contain developing gametes which will be deposited during the next breeding season. They will be termed the maturus junior and maturus

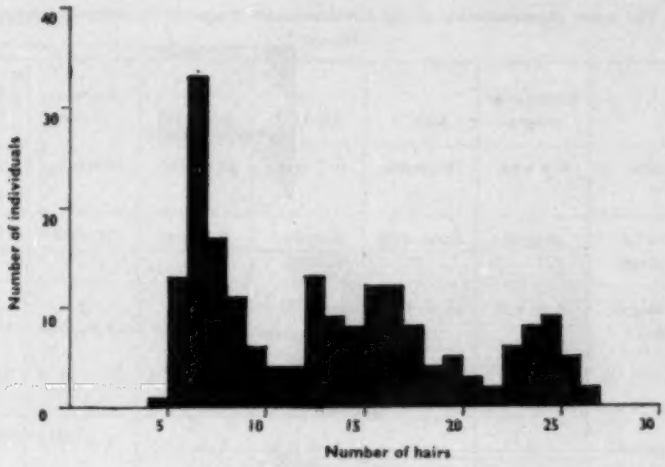

Fig. 6—Distribution of the number of hairs on the first genital sternite in male *S. maritima*. NB. The sample comprises adolescens III and maturus individuals only.

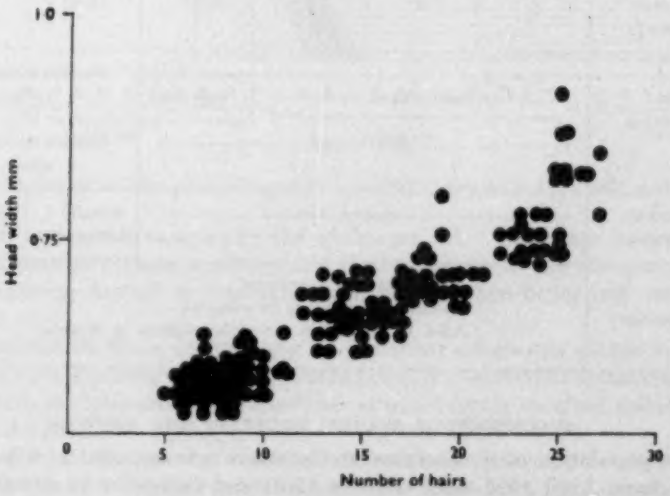

Fig. 7—A plot of the head width against the number of hairs on the first genital sternite in male *S. maritima*. NB the sample comprises adolescens III and maturus individuals only.

senior respectively to correspond with the nomenclature used by Verhoeff (1902–25) for *Lithobius forficatus* (L.) in which two maturus stages were also distinguished. The first three post-larval stages will be termed the adolescens I, II, and III following Verhoeff's terminology for *Mecistocephalus*. The main characteristics of the developmental stages of *S. maritima* are summarised in Table 2.

Table 2—The main characteristics of the developmental stages of *S. maritima* from Cuckmere Haven.

	Embryonic stages	Adol. I	Adol. II	Adol. III	Maturus junior	Maturus senior
Duration	6–8 wks.	10 mnths.	6–7 wks.	12 mnths.	12 mnths.	several years
Moults to next stage	August	June–July	August–Sept.	Autumn	Autumn	Autumn
Body length (mm.)	6·5–8·0	11·0–15·0	14·5–20·0	♂ 17·0–24·0 ♀ 21·0–27·0	♂ 24·0–29·5 ♀ 26·5–33·0	♂ 29·0–36·5 ♀ 31·0–43·0
Average no. coxal glands	0	1	3·5–5·0	♂ 6·5–10·5 ♀ 6·5–10·0	♂ 7·5–11·5 ♀ 7·5–13·0	♂ 7·5–13·0 ♀ 12·0–17·0
Hairs on genital sternite (♂)	0	0	2	5–11	11–21	22–27
Gonad ♀ condition	← undeveloped →			occas. with large ova	contain developing ova	
♂	← undeveloped →				contain developing sperm	
Seminal receptacle	← empty →				usually 1 whl. sperm	usually 2 whls. sperm
Behaviour	Peripatoid—no movement Foetus —writhing movements Adol. I —at first as foetus, as adult by August.					

SUCCESSION OF STAGES, MOULTING AND GROWTH

The population of *S. maritima* at Cuckmere was sampled at least once a month from April 1957 until October 1958, and thereafter bi-monthly until June 1959. The animals were sexed, weighed and assigned to an age group using the criteria described above. Figure 8 shows the percentage composition

of each year group of each sex at each collection. It was found that the pattern of the succession of stages was much the same each year.

Young adolescens I begin to appear in places other than the brooding habitats towards the end of August, and in June or July of the following year moult to the adolescens II stage, though a few individuals may moult a couple of months earlier. The adolescens II stage is a relatively short one and by

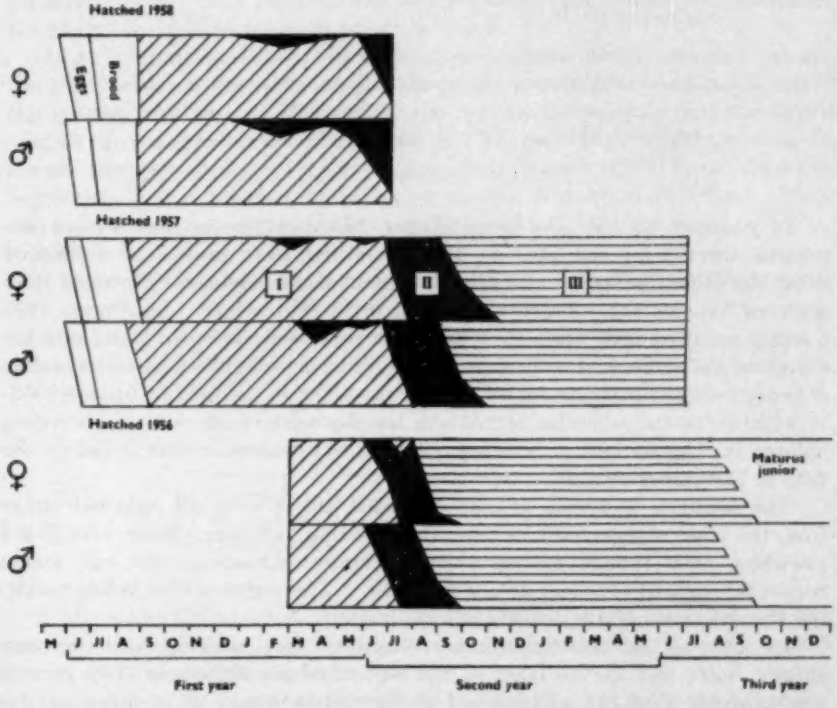

Fig. 8—Diagram of the succession of stages in *S. maritima* hatching in 1956, 1957, and 1958.

October is replaced in samples by the adolescens III. This stage passes some ten or so months without moulting and then gives rise to the maturus junior in the following August or September, the animals then being just over two years old.

The estimated times of moulting of the three adolescens stages may be confirmed by comparing them with moults actually observed in animals freshly brought into the laboratory, and the times at which newly moulted animals are found in the field.

Table 3 shows the dates on which ten adolescens *Strigamia* were found newly moulted in the field, or observed to moult in the laboratory. A comparison of this table with Fig. 8 shows, as might be expected, a very close correspondence between observed and predicted moulting times.

Table 3—Records of moults of adolescens *S. maritima* at Cuckmere Haven 1957–1959.

Moult	June	July	August	September
Adolescens I→II	1957 2 1959 2	1957 1♀	—	—
Adolescens II→III	—	—	— 1958 1♀	1957 1♂, 1♀ —
Adolescens III→ maturus	—	—	1957 1♀, 1♂	—

In contrast to the adolescens stages, maturus juniors and seniors are present throughout the year so their moulting time cannot be estimated using the same method as was used for the adolescens stages. However, two maturus females were observed to moult in the laboratory. In August 1957 a senior moulted and, while its head width increased, its coxal gland number remained the same, and in August 1958 a junior moulted to a maturus senior. A newly moulted maturus senior female was taken in the field in August 1957. In addition to this, exuviae of maturus females were common in the brooding habitat in August 1957. A newly moulted maturus male was found in the field in November 1957.

The fourteen moulting animals recorded above were all collected either from the main shingle bank or from the brooding habitats. None were found elsewhere even though extensive collections were made in the salt marsh region throughout 1958 and until June 1959. This suggests that before moulting the animals migrate into the shingle bank.

In order to test this hypothesis, collections were made in both the main shingle bank and at the edge of the salt marsh on 30th June 1959, since it was expected that the adolescens I of both sexes would be moulting at this time. The results are shown in Table 4. Since the adolescens III are immature, and do not moult until August, it was assumed that their distribution

Table 4—Composition of samples of *S. maritima* from the salt marsh and shingle bank at Cuckmere Haven on 30th June 1959.

Stage	Salt Marsh	Shingle Bank
Mature ♂	24	8
Mature ♀	15	0
Adolescens III	54	10
Adolescens II	6	39
Adolescens I	4	5

was typical of a non-breeding non-moulting group. In the calculation the adolescens I and II were massed since they belong to the same age group, and the ratio of the resultant group both in the salt marsh and in the shingle bank was compared with the ratio of adolescens III in the same habitats using the chi-square test. This showed the observed difference to be significant at the 0·1 per cent level (chi square=48·1).

The reason for this moulting migration may be that *Strigamia*, being of terrestrial origin, still requires non saline surroundings such as obtained at the top of the shingle bank, in which to moult.

If, as is highly probable, animals migrate up from the salt marsh to moult, the constitution of the population in the marsh should give some indication of the groups which are moulting, although it is always possible that the absent animals are engaged in some other activity. The most likely explanation of the almost complete absence of maturus males from the salt marsh from August to September is that they are moulting, as are the females at this time. They become common here again in October (Table 5).

Table 5—Composition of samples of *S. maritima* from the salt marsh at Cuckmere Haven during September and October 1958.

Stage	September	October
Mature ♀	2	9
Mature ♂	—	18
Adolescens III	1	6
Adolescens II	35	2
Adolescens I	14	5

If the males do moult in August and September at the same time as the females, then presumably the newly moulted male found in November 1957 was an atypical specimen in this respect.

Lawrence (1953) says that the split in the old skin of centipedes takes the form of a simple transverse fissure between the head plate and the first body segment, but this is certainly not the case in *S. maritima*. In this species the old cuticle ruptures along the frontal suture at the front of the head capsule just above the insertion of the antennae.

There do not appear to be any other descriptions of moulting in the Geophilomorpha, but Demange (1944) described the process in *Lithobius forficatus* in which the cuticle ruptures along the frontal suture as in *Strigamia*. Earlier workers had described the split as occuring behind the head, and Demange suggested that its location might vary in different species.

In Figure 9 the mean weights with their 95 per cent confidence limits are shown for the three adolescens stages and the maturus juniors of each sex and for each sample. The weights of the maturus seniors are not plotted since they are so variable, females varying from 18 mg. to 63 mg., and males from 16·5 to 43 mg., and probably comprise several year groups.

A considerable increase in weight accompanies each moult, the newly moulted adolescens II being almost twice as heavy as the adolescens I, the adolescens III more than half as heavy again as the adolescens II. The increment between the adolescens III and the maturus junior is much less than this. The short period between the moults from the adolescens I to II and the adolescens II to III may be necessitated by the rapid growth taking place at this time. An inter moult increase in weight takes place, though this is less marked. It can be seen in the adolescens I and III, but not in other stages. This is probably due to the small size of the samples. The maturus junior females show a drop in weight in late June ; this is to be expected since the females probably do not feed while brooding their young.

Although there is no detectable difference in the weight of adolescens I and II males and females, the adolescens III females appear to be slightly larger than the males, and in the maturus junior the difference is well marked, being even more accentuated in the maturus senior.

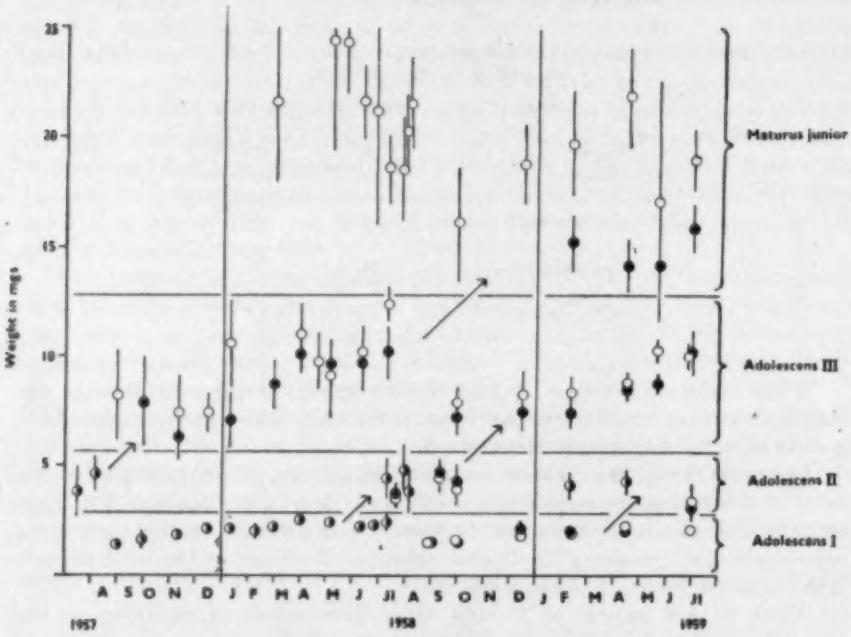

Fig. 9—Mean weights of adolescens and maturus junior *S. maritima* collected between July 1957 and July 1959 showing the 95 per cent confidence limits for the standard error of the mean. ● males ; ○ females ; ◑ mean using measurements for both sexes. Arrows link successive stages. NB maturus junior males were not distinguished from maturus seniors before February 1959.

REPRODUCTION

The anatomy of the male reproductive organs has been described for a number of geophilomorph species by several authors, namely Fabre (1855),

Schaufler (1885) and Tuzet & Manier (1953). In *S. maritima* the anatomy is essentially similar to that described for the other species. A pair of fusiform testes open by way of four vasa efferentia, one leaving from each end of each testis ; they fuse to form a much coiled vas deferens, in which the sperm are stored. The vas deferens divides two thirds of the way along its length to pass round the gut, and fuses again before opening ventrally on a sub-terminal penis. Two pairs of tubular accessory glands lie alongside the gut and open posteriorly into the genital atrium.

Examination of both temporary and permanent mounts, the latter stained in borax carmine after Bouin fixation, showed that in maturus males there is a gradual build up of sperm in the vas deferens beginning in August, when small groups of sperm appear at intervals along its length. The sperm are about 2 mm. long and gradually become rolled up in the distal region of the vas deferens, and lower down are coiled on themselves in the manner of a clock-spring. By January the vas deferens is much distended with sperm, and remains thus until May when the number of sperm diminishes. By mid June the vas deferens is again empty.

The anatomy of the female reproductive system of *S. maritima* resembles closely that described for other genera by Fabre (1855) and Schaufler (1889), consisting of an unpaired tubular ovary leading to a short oviduct which divides to pass round the gut, fusing again to open ventrally into the sub-terminal genital atrium. There is a short pair of accessory glands, and in the prepenultimate pediferous segment there is a pair of spherical receptacula semines the convoluted ducts of which open into the genital atrium along with the accessory glands.

The growth rate of oocytes was determined by measuring the egg width in permanent preparations stained in borax carmine after Bouin fixation by means of a micrometer eyepiece. Usually about twenty-five ova were measured from between three and six individuals each month, but preparations of near ripe females were sometimes difficult to clear adequately and in these the size of small oocytes was consequently difficult to determine. In preparations of the smaller adolescens stages fewer than twenty-five oocytes could be measured.

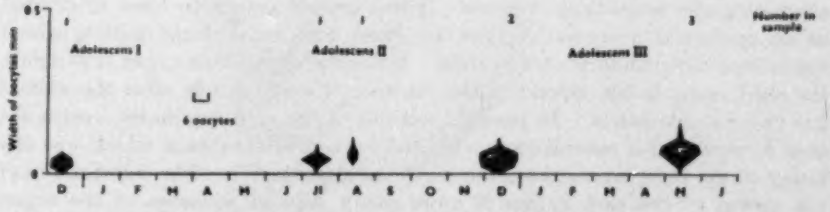

Fig. 10—Widths of oocytes in adolescens female *S. maritima*.

There appears to be a slow increase in the size of the oocytes in the ovary during the first three adolescens stages (Fig. 10), but at the end of the autumn in which the maturus junior is produced, the rate of increase is accelerated, and by October it is obvious that the ovary contains eggs of two sizes, the

larger consisting of oocytes which will be laid the following summer, the smaller presumably in subsequent years (Fig. 11). There is no evidence that the rate of growth of the oocytes in the maturus senior differs from that in the maturus junior, except in a small percentage of maturus juniors which do not lay eggs. Occasionally large adolescens III are found with ovaries in which the oocytes fall into two size groups, suggesting that they will lay eggs before

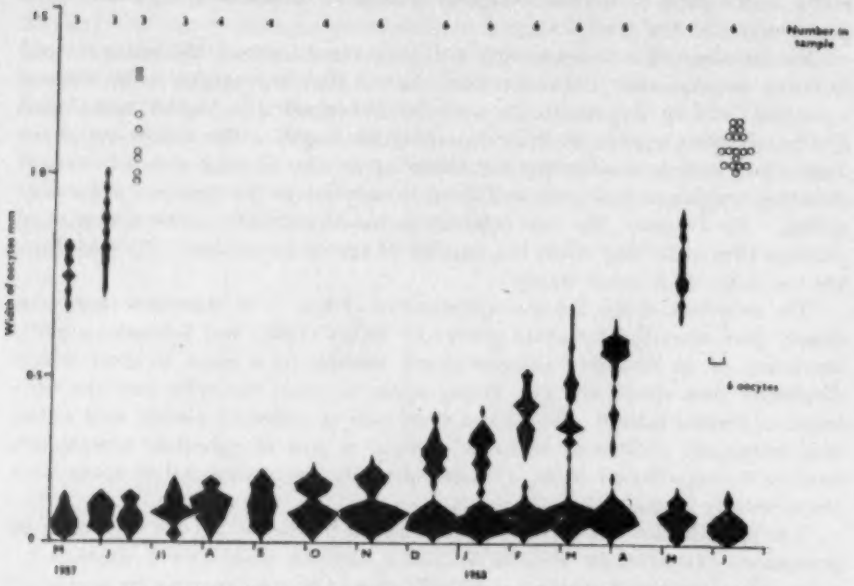

Fig. 11—Widths of oocytes in maturus females (kites), and of eggs (open circles).

they reach the maturus junior stage. In June 1958 an adolescens III female was found with a clutch of three eggs ; the ovaries of adolescens III females have never been found to contain more than five oocytes of the larger size group.

Whether or not the females have been inseminated may be determined by examining the seminal receptacles. These organs appear to have originated as an epidermal invagination and are lined with unhardened cuticle which stains blue with Mallory's triple stain. Since the duct of the organ is so small, the shed lining is left behind in the lumen of the receptacle after the animal has cast its old cuticle. In paraffin sections of the maturus junior, sperm are seen to surround a central cavity limited by a fine membrane which was the lining of the receptaculum seminis of the adolescens III. The relationship of the sperm to the cast linings is more easily seen in squashes of the organ (Figs. 12 and 13). In adolescens III females the receptaculum contains only one cast lining and this is very small and delicate. In the maturus junior two linings are visible, the second much larger than the first which it surrounds. In the maturus senior a third lining, that of the receptaculum of the maturus junior is not always apparent, probably because it is ruptured during squashing when the whorls of sperm burst through it.

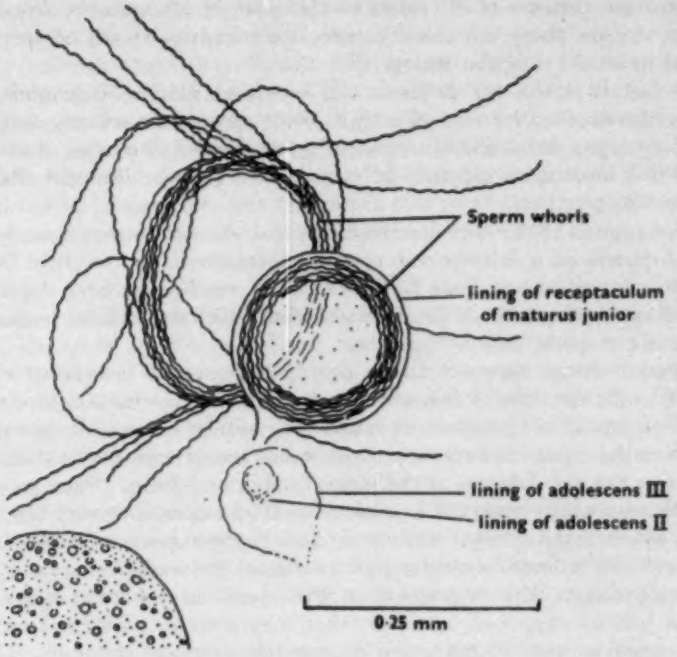

Fig. 12—Drawing of a squash of a receptaculum seminis of a maturus senior female *S. maritima*.

Fig. 13—Stereogram of a receptaculum of a maturus senior female *S. maritima*.

Sperm are present at all times of the year in all maturus females ; in maturus seniors there are usually several concentric whorls of sperm each enclosed in an old cuticular lining.

The fact that the vas deferens and accessory glands of geophilomorphs open to the exterior by way of a protrusible structure variously termed the copulatory organ, intromittent organ, or penis, has led a number of authors to assume that centipedes copulate but this has never been observed (Palmen & Rantala 1954).

As long ago as 1855 Fabre described how male *Geophilus convolvens* deposited spermatophores on a delicate web spun by themselves, and recently Demange (1956) has described how male *Lithobius piceus gracilitarsus* Brol. deposit their spherical spermatophores on similar webs from which the females remove them with their gonopods.

Spermatophores were not found during the present investigation, so an attempt to fix the time of insemination by regular examination of the reproductive organs of both sexes was made. It seemed reasonable to expect an increase in the number of sperm in the seminal receptacles at the time of their decrease in the vas deferens of the males in May and June. Such an increase would be most easily observed in adolescens III females since very few of them contain sperm in the spring. Only two of the thirty-two examined in May 1959 were fertilised, whereas maturus juniors almost invariably contain sperm in their receptacles. The disposition of the sperm in the receptacles of the maturus juniors suggested, however, that they were not fertilised until they had moulted to this fourth stage during the previous autumn. This was confirmed by examining ten adolescens III females in July 1959 a month after the males had lost their sperm. None of the females had been inseminated.

Observations made at Cuckmere during May and June suggested that maturus males migrated up the beach at the time during which the vas deferens became emptied of sperm. On 21st May 1958 a sample of animals from the brooding habitat consisted of seven maturus males and ten maturus females, on 9th June a sample from the same habitat yielded fifteen females, three adolescens, but no males. A comparison of the proportion of males to females on these two dates using the chi square test shows them to be significantly different at the 1 per cent level (chi square = 7·07).

Since this upshore migration by the males corresponds with the emptying of their vasa deferentia, it would seem reasonable to suppose that the purpose of the migration is to deposit the spermatophores near the maturus females, there being no other obvious explanation.

It seems probable then that the maturus females are fertilised in May before laying their eggs, but the newly recruited maturus juniors are fertilised soon after the moult which produces them in August. In August, the vasa deferentia of the males contain only small quantities of sperm. This may not mean that the rate of sperm production is low, for the quantity of sperm in the reproductive tract will depend on the rate of removal as well as the rate of production. The accumulation of sperm in the vas deferens during the winter may be due to a slowing down or complete cessation of spermatophore production at that time.

The evidence suggests that the mature males produce spermatophores both in autumn and in spring. In May some at least of the males migrate to the brooding habitats where they deposit their sperm. In the autumn the males migrate into the main shingle bank to moult at the same time providing spermatophores for the newly moulted juniors. Palmen & Rantala (1954) showed that female *Pachymerium* collected in the autumn can raise broods successfully the next spring and concluded that in these cases fertilisation occurred before hibernation, but they state that in the majority of specimens fertilisation seems to occur after hibernation.

LENGTH OF LIFE

Lawrence (1953) quotes figures for the life span of various cryptozoic groups (isopods, chilopods and Onychophora), and suggests that many of them " end their life by natural death from old age ". He gives no figures for geophilomorphs, but quotes Verhoeff's (1902–25) figures of five or six years for *Lithobius*. The only figures for geophilomorphs appear to be those of Palmen & Rantala who concluded from the size of brooding females that the largest were in their third summer or " possibly even still older ".

In order to age maturus seniors exactly, a character which continues to increase with age is required. Coxal gland number and hairs on the genital sternite are of no use in this respect. An analysis of head width data using probability paper is applicable, but the numbers measured were not sufficient for this to be done.

A character which continues to change in the maturus female is the number of whorls of sperm in the seminal receptacle. Figure 14 shows a plot of the number of whorls against head width which is here used as a measure of size. It can be seen that the larger animals tend to have a greater number of whorls than smaller ones. Maturus juniors, which usually have only one whorl of sperm in their receptacles are between two and three years, and therefore females with three whorls must be between four and five years old. Two individuals shown in Fig. 14 have four whorls of sperm and it is probable that there were more individuals in this group. Unfortunately, it becomes increasingly difficult to count the whorls in squashes as their number increases, and a number of cases where it was not possible to be certain whether there were three or four whorls present were omitted from the graph. Females with four whorls are in all probability between five and six years old.

No method of separating age groups of maturus males was found although two changes correlated with age were observed. In six of the 750 specimens of all post larval stages of both sexes dissected the mid gut instead of being the normal orange-brown colour was violet. All six specimens were maturus males. Needham (1960) describes a mauve-pink pigment from young *Scolioplanes* (=*Strigamia*) *maritimus* which is very similar to the lithobioviolin of *Lithobius forficatus*. The colouration is probably due to the deposition of this pigment. The pigment is also visible in the mid gut of larvae when it is a mauve-pink colour.

A second characteristic of maturus males is the reduction in the variation in the number of hairs on the genital sternite. Figure 6 shows that although

the variation increases from the adolescens III to the maturus junior, the variation in number is about half as great in the maturus senior as in the maturus junior. Presumably there is either compensation when the new setae are formed in the pre moult maturus junior, more being formed in specimens with a low setal number, less in those with a high one, or more likely the extremes are selected against.

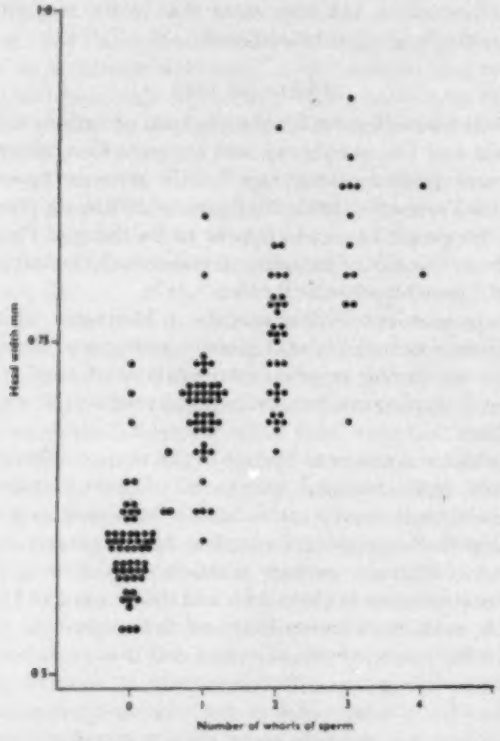

Fig. 14—A plot of the number of whorls of sperm in receptacula seminea against head width in female *S. maritima*.

FECUNDITY AND MORTALITY

In practice it was found very difficult to collect all the eggs from a given brooding female because eggs were lost when the brood cavity was exposed. Fecundity was therefore calculated from the number of large oocytes in the ovaries of mature females collected in the winter and spring. This was considered to be justifiable since no evidence of resorption of ova was found. The numbers of eggs found by this method varied from three in an adolescens III to forty-four in a large maturus senior. The average number of eggs calculated from forty maturus juniors was thirteen, and from thirty-two seniors, twenty-seven.

The sex ratio calculated from adolescens I collected between June 1958 and April 1959 was 117 males to 110 females and from adolescens III collected between March 1957 and April 1959, 240 males to 225 females. The sex ratio amongst maturi is more difficult to determine, since for much of the year the two sexes are differentially distributed. However, samples taken from the edge of the salt marsh in February and April 1959, when such a differential distribution was unlikely, gave a ratio of forty-six male to forty female maturus juniors and twenty-two male to twenty-three female maturus seniors. Thus a sex ratio of approximately unity is maintained in all age groups.

Of the 244 maturus females collected between October 1957 and April 1959, 143 were juniors, 101 seniors. Using this ratio and that for the average number of eggs for each stage, an average egg number of 18·8 per mature female per year is obtained. Considering that the females have to survive for two years before they produce young, the fecundity of *Strigamia* is very low.

PREY AND FEEDING

Several workers have made observations on the prey of *S. maritima*. Pocock (1900) found the species feeding on a crustacean, but his account does not make it clear whether this was a woodlouse or an amphipod. More recently, Blower (1957) described how *Strigamia* at Port Erin in the Isle of Man feed on the barnacle *Balanus balanoides* (L.) and the periwinkle *Littorina saxatilis* (Olivi) at night. Similarly, Turk & Turk (1958) have observed the species feeding nocturnally on barnacles, winkles and top shells at Mount Edgecumbe in Cornwall.

At Cuckmere, collections were made in the region of the berm of the main shingle bank in October 1956 and monthly from April 1957 to May 1958. The most common animals here were the talitrid amphipod *Orchestia gammarella*, enchytraeid worms, the beetles *Cafius xantholoma* Gr. and *Trechus fulvus* (Dej.), the spider *Lycosa arenicola* O. P.—Cambridge, and various small mites and Collembola. During this time *Strigamia* was only twice found feeding. On 30th October 1956 a large male was seen with an enchytraeid in its jaws, and on 10th November 1957 another specimen was found feeding on an *Orchestia*.

The population at the edge of the salt marsh was sampled at one or two monthly intervals from June 1958 to June 1959. In this region the commonest animals were *Orchestia gammarella*, the isopod *Sphaeroma* sp., the beetle *Pogonus chalceus* Marsh, and the gastropod *Potamopyrgus* (*Hydrobia*) *jenkinsi*. Immature linyphiid spiders were common in the summer months, but small forms such as Collembola and mites were rare. *Strigamia* was found feeding once on *Orchestia* when on 30th April 1959 about twenty specimens were found feeding on one individual, and on *Sphaeroma* on thirty-five occasions (Table 6). All post larval stages of the centipede were found feeding on this isopod, either singly, or in groups of up to twenty individuals. In most cases the crustacean was attacked through its ventral surface, presumably because its integument is much thinner and more easily penetrated here. On seven occasions the centipedes were found imprisoned by their heads or antennae having been

Table 6—Observations on predation on *Sphaeroma* by *S. maritima* at Cuckmere Haven 1958–1959.

Date	Number of observations	Number of *Strigamia* feeding	Site of entry	Abundance of *Sphaeroma*
1958				
June 9th	8	6 singly 2 pairs	3 dorsal 5 ventral	v. abundant
July 7th	7	10	6 dorsal 1 ventral	v. common
August 6th	—	—	—	rare
Sept. 1st	—	—	—	uncommon
Oct. 6th	—	—	—	absent
Nov. 16th	1	several	ventral	rare
1959				
Feb. 19th	1	1	ventral	fairly common
April 14th	8	5 groups 3 singly	ventral	common
April 30th	4	4 singly	ventral	no observation
May 23rd	—	—	—	v. common
June 30th	6	1 group of 15–20 4 groups of 6 or 7 1 singly	ventral	v. common

trapped when the *Sphaeroma* rolled into a ball presumably on being disturbed by me.

In June and July 1958, nine of the fifteen specimens on which *Strigamia* was found feeding were attacked by way of their dorsal surfaces. Examination of these animals in the laboratory showed that those attacked dorsally had cracked or partially crushed terga. Such damage could not be inflicted by the centipedes, nor by wave action, for the salt marsh is sheltered from the sea by the main shingle bank. It may, however, have been cause by the pressure exerted by holiday makers walking over the shingle which is very shallow at the edge of the marsh. In 1959 holiday makers visited the area less frequently since the shingle bank had been bulldozed during the winter making it much steeper in this region. It may be significant that no damaged *Sphaeroma* were found in 1959, nor were any *Strigamia* seen entering the isopod dorsally.

No plant or animal remains were found in seven hundred and fifty mounts of the guts of animals collected throughout the period of the investigation and comprising all the post-larval developmental stages, nor in the faeces produced by these animals prior to their dissection. Lawrence (1953) observing the

narrow oesophagus and small mouthparts of geophilomorphs suggested that they might be suctorial feeders ; the above observation supports this suggestion.

The centipede's method of feeding was investigated in the laboratory by placing it in a plastic pill box lined with damp filter paper with either *Orchestia*, *Sphaeroma*, or *Drosophila*.

Two sizes of *Orchestia* were used, small individuals between 2 and 4 mm. in length, and large ones measuring a centimeter or more. The former were frequently attacked and the bodies left in a dismembered state, the latter were only attacked if damaged or dying. On one occasion the centipede's method of opening *Orchestia* was observed when a large dying specimen of the amphipod was attacked. The centipede possesses a pair of poison claws on the first trunk segment, these move laterally, while the fused coxopodites which carry them move in the dorso-ventral plane. The centipede in opening the *Orchestia* levered up a tergite with its poison claws, and, turning its head on one side, pierced the intersegmental membrane with one of these, sliding it round the tergite thus making a transverse slit in the amphipod's body. It then pushed its head and anterior segments into the body cavity and could be seen through the animals semi-transparent integument. The centipede's poison claws moved continuously macerating the body tissues ; the movement of fat droplets showed that there were irregular currents to and from the head of the centipede. These were probably due to the expulsion of digestive fluids and the sucking in of the liquid products of digestion. Blower (1957) remarked that the slimy consistency to which the soft parts of barnacles were reduced by *Strigamia* might be due to extraintestinal digestion.

Small *Orchestia* and *Drosophila* were seized with the poison claws and held with these and the first seven or eight pairs of walking legs during feeding, the anterior end of the centipede being arched over the prey. Whereas these small animals which have relatively soft integuments, were reduced to very small pieces, the harder skeletoned *Sphaeroma* and large *Orchestia* were left with their skeletal elements intact.

Brade-Birks (1929) in his review of feeding in centipedes concludes that the geophilomorphs though normally carnivorous might sometimes take plant food. *Strigamia maritima* appears to be a non-specific carnivore and has now been reported to feed on barnacles, winkles, top shells, isopods, amphipods and enchytraeids as well as on *Drosophila* in the laboratory. It probably takes most animals whose integuments it can penetrate with its prehensors. No evidence of a herbivorous diet was found in this species. *Strigamia* obviously feeds commonly in the daytime as well as nocturnally as reported by Blower and Turk & Turk.

The group feeding observed in the present investigation and also by Blower presumably results from the high population density and mobility of the species. Such group feeding is probably advantageous in that it makes prey, which would otherwise be invulnerable, available to small specimens.

PREDATORS AND PARASITES

In the absence of any field observations on the predators of *Strigamia*, laboratory experiments were carried out using the more common predatory

species found at Cuckmere. These were placed in plastic pill boxes lined with damp filter paper with various stadia of *Strigamia*, and examined at daily intervals thereafter.

The carabid beetle *Pogonus chalceus* readily accepted adolescens I *Strigamia* grasping the centipede's posterior end and eating it from behind, gradually working forward. The adolescens III stage was never eaten, although beetles were sometimes observed to make futile grabs at these larger specimens. Similarly, the staphylinid beetle *Cafius xantholoma* only successfully attacked adolescens I *Strigamia*. Neither *Lycosa arenicola* nor immature linyphiid spiders accepted the centipede.

No parasites were found in the 750 specimens dissected from Cuckmere, neither were any visible in the borax carmine stained preparations of their guts. None were seen in some twenty squashes of the gut of the centipede, nor in four sets of serial sections of the animal.

Verhoeff (1902–25) lists four geophilomorphs which have been found to be parasitised by Sporozoa, but as yet it is not known how frequently terrestrial species are infected by these parasites. It is possible that the absence of such parasites from *S. maritima* may be due to the unsuitability of the habitat it occupies for the development of the stages of the Sporozoa which are passed outside the centipede.

THE DISTRIBUTION OF *STRIGAMIA* AT CUCKMERE

During the course of the investigation *Strigamia* was found only in those parts of the shingle bank where the air was saturated. This was to be expected, since most centipedes possess very incomplete water-proofing mechanisms (Blower 1955 b). Several requirements, namely the probable need of non-saline conditions for successful moulting and egg laying, and the necessity for males to deposit their spermatophores near the females, which cause different patterns of distribution in this habitat at different times of the year, have already been described above.

In addition to these, there are a number of other factors, which appear to affect the distribution of *Strigamia* in the habitat. Small animals were sometimes very common at a given point whereas large specimens were relatively rare. The most marked example of this was observed in April 1959, when adolescens I were proportionately far more numerous beneath *Obiene* drift on the shingle bank above the salt marsh than they were at the edge of the salt marsh (Table 7). A chi square test shows the difference in the ratio of adolescens I and II to adolescens III at the edge of the salt marsh, and beneath the *Obiene* drift to be significantly different at the 1 per cent level (chi square = 28·3). A difference in food requirements probably accounts for this, since small worms and arthropods which would form a suitable food for the adolescens I were common in the *Obiene* drift, but absent from the salt marsh.

During high tides, when the sea seeps through the shingle bank flooding the salt marsh, many centipedes become submerged and then tend to migrate up out of the area. This appeared to be the case on 25th April 1959, when the effect of a high spring tide was exaggerated by a strong onshore wind. At 1 p.m., the salt marsh pool, although at its lowest that day, was still about a foot above

Table 7—Composition of samples of *S. maritima* from the salt marsh and from *Obiene* drift at Cuckmere Haven April 1959.

Stage	Salt Marsh	Drift
Mature males	22	14
Mature females	20	13
Adolescens III	49	47
Adolescens I & II	20	131

its normal level due to the slow rate of drainage of the pool after having been filled by the previous evening's tide. The centipede was found to be present in considerable numbers in the shingle one foot above the water's edge. Four samples of shingle of approximately 5,000 ccs. each were taken from this region, and the number of centipedes found in them by hand sorting compared with the number found in four similar samples taken at exactly the same level at

Table 8—Number of *Strigamia* present in four 5,000 cc. samples of shingle taken from the same level of the main shingle bank at Cuckmere Haven at Spring tides (25th April) and Neap tides (30th April).

Date	Sample number			
	1	2	3	4
25th April 1959	10	14	28	11
30th April 1959	1	9	8	2

neap tides five days later (Table 8). The number of animals present at springs was significantly greater at the 5 per cent level than the number at neaps when tested using the " Student "s' t test.

During the course of collecting on 28th January 1959 it was noticed that *Strigamia* was particularly common on the seaward, south facing side of the shingle bank just below the berm. It occurred some two inches below the surface at the boundary of wet and dry shingle. The temperature here, measured with a mercury thermometer was 12°C., which was seven degrees higher than the temperature on the north facing slope of the bank, and nine degrees higher than the shingle temperature at the depth of one foot. The fact that the shingle heats up rapidly will be of advantage in winter since it provides conditions in which the centipedes may be active, and in this particular case the site of such rapid heating is an advantageous one since there is a plentiful fauna supported by the organic matter deposited at the berm by the tide.

DISCUSSION

Strigamia maritima is the only member of its genus known to occur on the sea shore, the other species of the genus being woodland species. Judging from the results obtained at Cuckmere Haven, the species is probably fairly

mobile, concentrating in areas of the shingle bank which are climatically favourable and have a good food supply. It is found only at the top of the shore, around the High Water Spring drift line on the seaward side of the shingle bank, but this may well be due to the unstable nature of this part of the beach, and the fact that the fauna is usually sparse here : Blower (1957) has found the species in coarse shale down to mid-tide level at Port Erin. The animal is found far lower down on the landward side where it feeds on *Sphaeroma* and is liable to spasmodic immersion at high spring tides and during bad weather, but it is difficult to equate conditions here with those prevailing at a particular tidal level. Immersed animals migrate up the beach suggesting that even the adults are not well adapted to resist fully marine conditions for long.

Mature females migrate into moist sandy areas where conditions approximate to terrestrial ones to lay their eggs, which are very permeable, and to brood their young which are rapidly desiccated in unsaturated air. The time of egg laying appears to be geared to correspond with the time when spring tides have their smallest amplitude and with the least stormy part of the year, and is far better defined than in terrestrial species. This gearing is reflected in the size of the oocytes in the ovary, which varies only very slightly between different individuals at a given time of the year. Males also migrate up the beach to deposit their spermatophores, and all stages migrate into the top of the shingle bank to moult. Such migrations suggest that the spermatophores, eggs, larval stages and moulting animals are unable to withstand much immersion in sea water.

It is possible to separate five post-larval instars in both sexes, the last two being mature as they are in lithobiomorphs. Maturus females may be aged by counting the number of whorls of sperm in their seminal receptacles, the number increasing by one whorl each year. Since the cast receptacular linings remain intact with their openings facing the receptacular duct, sperm passing up this duct are likely to find their way into the old linings and will therefore become arranged in a number of whorls.

The centipede which is a non-specific carnivore feeds at least from February to November. The main food source at Cuckmere is *Sphaeroma* probably because this crustacean is so abundant. It seems that a number of *Sphaeroma* were particularly vulnerable to attack in the summer of 1958 because of their damaged exoskeletons : the damage was probably caused by the pressure exerted by holiday makers walking over the shingle in this region.

The absence of internal parasites may be due to the unsuitability of the environment for the development of their intermediate stages. The only likely predators of *Strigamia* are carabid and staphylinid beetles, and these only take the smaller stadia. The absence of parasites and large predators probably account to a large extent for the low fecundity of this species.

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SUMMARY

The habitat of the littoral centipede *Strigamia maritima* (Leach) at Cuckmere Haven, Sussex is described. The densest aggregations of the animal are found where, on its landward side a shingle bank forming the upper part of the shore meets a salt marsh. *Sphaeroma* is the centipede's chief source of food in this locality. Animals migrate from this region when immersed at very high tides by water seeping through the shingle bank. Females also migrate from the marsh to the non saline region of the shingle bank before laying their eggs which are very permeable and shrink rapidly in sea water. The time of egg laying is far more restricted than in terrestrial species and takes place at the time of the year when there is least danger of flooding from high tides.

The methods used to distinguish three larval and five post-larval stages are described. Both sexes mature at the fourth post-larval stage which is reached in just over two years after hatching. Before moulting animals migrate to the top of the shingle bank.

In males there is a gradual build up of sperm from August until the following May when the sperm are discharged. In females the eggs commence their growth in the summer and are laid the following June. Insemination probably takes place twice a year, the mature females being inseminated in May before egg laying, older immature females being inseminated in the autumn.

Mature females can be aged by the number of whorls of sperm in their seminal receptacles, and this shows that some females live for five years. The sex ratio in all stages is unity, and as is to be expected of a carnivore with no parasites and probably few predators, the fecundity is low ; the average number of eggs laid per year per mature female being 18·8.

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THE FORM AND FUNCTION OF THE FORE-GUT IN ANURAN LARVAE (AMPHIBIA, SALIENTIA) WITH PARTICULAR REFERENCE TO THE *MANICOTTO GLANDULARE*

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(With 6 plates and 13 figures in the text)

The structure and development of the larval fore-gut is described in *Rana ridibunda*. It is shown that the *manicotto* is formed from elements of the ventral pancreatic anlage which proliferate within the limits of the gut-wall. The pattern of the *manicotto* is examined in seventy-five anuran species representing thirty genera and nine families. Three main deviations from the typically ranid plan are described: (*a*) persistence of a *manicotto*/pancreatic bridge until late metamorphosis (e.g. in *Heleophryne rosei*); (*b*) restriction of *manicotto* tissue to the pancreatic half of the gut tube (*Alytes obstetricans*) and (*c*) concentration of the gland into a discrete, muscular fore-gut diverticulum (*Philautus gryllus*). In (*b*) and (*c*) specialized mechanisms for sorting gut contents are also described. Defined regions of the fore-gut are examined for enzyme action. It is shown that the *manicotto* functions proteolytically at ≒ pH 7·8. No such activity is detectable in any other part of the fore-gut and tests for amylase and lipase are negative for all regions. Peptic action is first detectable about two days before the end of metamorphosis, approximately six days after the cessation of *manicotto* activity. The effect of the chemical nature of the type of food taken during ontogeny is considered. It is shown that the nature of the diet has no detectable effect on either the form or the physiology of the fore-gut. The distribution of the *manicotto* is reviewed. It is concluded that the occurrence of the gland is neither a correlate of phylogenetic relationship nor of feeding habits but that its presence results from the protraction of the early anlage stage in the development of the larval pancreas. It is suggested that the absence of a peptic digestion in anuran larvae reflects a need for chloride ion economy in these forms.

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INTRODUCTION

Relatively few investigations have been specifically concerned with the form and function of the fore-gut in larval Anura and the observations which

are available are restricted to three genera : *Rana* (six species), *Bufo* (two species) and *Alytes* (one species). Authors disagree concerning the stage at which gastric differentiation occurs : some (Ratner, 1891 ; Reuter, 1900 ; Bowers, 1909 ; Janes 1934 ; Kaywin, 1936) refer to a definite larval stomach but others (Swingle, 1918 ; Blacher & Liosner, 1930) contend that a stomach is not developed until the end of metamorphosis. Barrington (1946) studied tadpole stages of *Rana temporaria* and *Bufo bufo*. In the former he recorded a glandular, stomach-like swelling of the fore-gut immediately posterior to the oesophagus but found no such structure in the toad larvae. Barrington emphasized the need to consider function as well as form before assigning a gastric role to any region of the alimentary canal and, since his experiments showed that extracts of tadpole fore-guts give negative reactions when tested for peptic activity, he concluded that the fore-gut expansion of the *Rana temporaria* larva is not a true stomach. This view, based on physiological evidence, supports that of Lambertini (1928) who, from purely morphological considerations, concluded that the fore-gut swelling of *Rana temporaria* tadpoles is essentially a larval structure, for which he proposed the descriptive name of *manicotto glandulare*.

Lambertini offered no evidence concerning the function of the *manicotto*. Barrington's physiological analysis made him " doubtful whether the organ is zymogenous " and led him to conclude that " for the present..... the exact function of the organ must remain in doubt ". Uncertainty also attends the relationship of the gland to the definitive stomach. Duesberg (1906) states that the *manicotto* elements persist unchanged from the larva to the adult. Marcelin (1903) asserts that gland formation is a process of progressive elaboration which begins in the early tadpole stage and proceeds antero-posteriorly from the gastro-duodenal junction up to, and including, the oesophagus. Kaywin (1936) and Barrington (1946) conclude that the larval glands de-differentiate and then become reorganised to form the glands of the adult stomach. Lambertini (1928) and Janes (1934) consider that the *manicotto* units atrophy at metamorphosis and that the adult glands arise as new developments from the reconstituted epithelium. Janes, tentatively, further suggests that *some* contribution to the post-metamorphic stomach may be made by the larval glands.

The present investigation was undertaken to study the form, function and development of the gland ; the fate of its secretory elements ; its distribution within the Order and the modifications (if any) which it, and the larval fore-gut as a whole, undergo in different species.

MATERIAL AND METHODS

Most of the material used in studying the structure and distribution of the *manicotto* was received already fixed. *Rana ridibunda*, *R. temporaria*, *Bufo bufo* and *B. calamita* larvae were collected in the field and those of *Xenopus laevis* were reared from eggs laid in the laboratory. Tadpoles series of the above species were fixed in Clarke's solution, Helly's fluid, Susa or Zenker without acetic. Heidenhain's iron haematoxylin (with Orange G) and Azan techniques were the chief staining methods employed. Use was made, also, of toluidine

blue, thionine and Southgate's mucicarmine for mucus. In species whose larvae were available alive, the distribution of cilia was determined by three methods : (*a*) by following the movements of fine carbon particles placed on the epithelia of freshly teased fore-guts ; (*b*) by teasing fresh preparations of selected regions in a dissociating medium consisting of 0·002 M Versene (disodium salt of ethylenediamine tetra-acetic acid) in Stearns (1955) solution, from which calcium and magnesium salts had been omitted—this technique enables the free border to be examined whilst the cell is still alive ; (*c*) by reference to sectioned material of which that stained by the iron haematoxylin and Azan procedures proved particularly useful. In cases where fixed material only was available, conclusions concerning distribution of cilia were based entirely on histological evidence.

The methods employed for enzyme analyses etc. will be described in the appropriate sections.

THE STRUCTURE OF THE LARVAL FORE-GUT OF *RANA RIDIBUNDA*

Barrington (1946) was the first to recognize the fundamental distinction between the fore-gut and the intestine (mid-gut) in larval Anura. He separated the two regions according to the histological nature of their epithelia, the latter being characterized by " ' striated border ' cells of the intestinal type, together with scattered goblet cells " and the former by possessing " a ciliated and mucus-secreting epithelium ". These criteria were found valid in all the species examined during the present investigation (Table 1) ; contrary to the reports of Marcelin (1903), Janes (1934) and Kaywin (1936) cilia were not found extending into the intestine proper except for a few species which possess a narrow ciliated ring around the opening of the hepato-pancreatic duct. This ring is too localized and the nature of the surrounding epithelial cells too distinctly intestinal to invalidate the general applicability of Barrington's definition. The following description applies to the tadpole of *Rana ridibunda* and will form a basis for discussing the variations occurring in other species.

The fore-gut extends back from the transverse septum towards the liver and the pancreas. The point at which the fore-gut terminates and the intestine begins cannot be determined by gross dissection. Histologically, it can be seen that the transition occurs immediately posterior to the point where the gut loops around the pancreas on the animal's right-hand side, ventral to and partially enclosed in the liver lobe. At the apex of the pancreatic loop, the fore-gut expands into a pink, muff-like structure (Fig. 1). This is the *manicotto glandulare* of Lambertini.

The fore-gut epithelium consists of mucus-secreting columnar and goblet elements (the former being by far the more numerous) interspersed with ciliated cells. At the base of these cells lie large, rounded units (the "giant cells" of Reuter, 1900: the "basal cells" of Duesberg, 1906) which become more numerous and noticeable towards metamorphosis. As will be shown later, these cells have a significant relationship with the lining of the definitive, post-metamorphic gut.

Barrington (1946) concluded that the *manicotto* epithelium of *Rana temporaria* is devoid of ciliated elements and consists only of mucus-secreting cells. In none of the ranid species examined during the present investigation was a completely cilia-free *manicotto* encountered. The epithelial pattern of the *manicotto* in *Rana ridibunda* differs from that of the general fore-gut only in being invaded and ruptured by the *manicotto* glands (*vide infra*). Ciliation

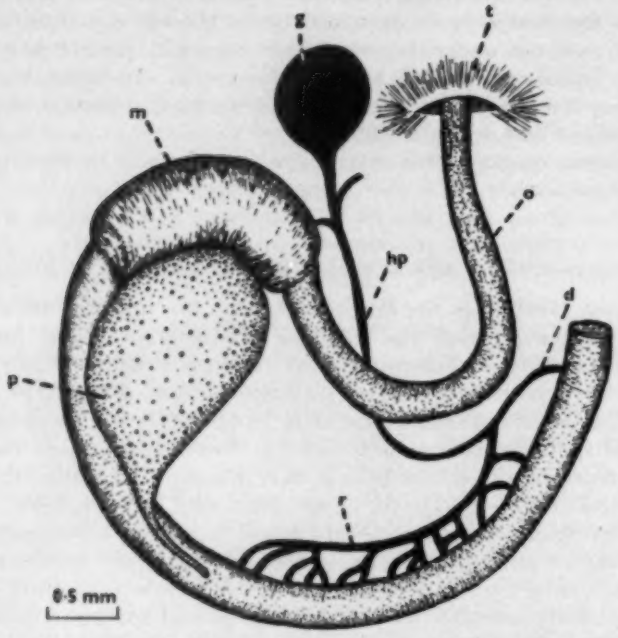

Fig. 1—*Rana ridibunda*. Ventral view of the larval fore-gut. (Stage III, Taylor & Kollros). (Key to lettering, p. 282).

extends continuously from the oesophagus up to the proximal gut loop where the fore-gut bends obliquely towards the liver (Fig. 2 A and F ; Pl. 1, fig. 1). At this point ciliation is restricted to a narrow tract on the outermost side of the loop. This tract progressively narrows and terminates soon after the completion of the proximal loop (Fig. 2A and F). The epithelium then continues unciliated until a second narrow tract leads round the outermost side of the second loop and into the *manicotto*, where ciliation extends over the epithelial surface as a whole. From the *manicotto* a third ciliated tract runs for a short distance round the distal limb of the second loop in the direction of the mid-gut (Fig. 2, A-F). The ciliated tract of the proximal loop represents the morphologically dorsal and the *manicotto* approach and departure tracts the morphologically ventral sides of the gut tube. Their present position is due firstly, and chiefly, to the rapid growth in length of the gut within the restricting abdominal cavity and, secondly, to the displacement factor introduced by the liver. The functional significance of the tracts, probably, is

to prevent accumulation and stagnation of food particles at these points. The angles of the fore-gut loops are much more abrupt than those of the intestinal spiral and their outermost margins, where the ciliated bands occur, represent the most likely sites at which food particles might be side-tracked from the main gut stream. Ciliation is clearly essential in the *manicotto* not only because, in the definitive gland, its epithelium is discontinuous and provides particularly dangerous stagnation points, but, also, because its muscular coat is so attenuated as to be functionally useless in the movement of gut contents

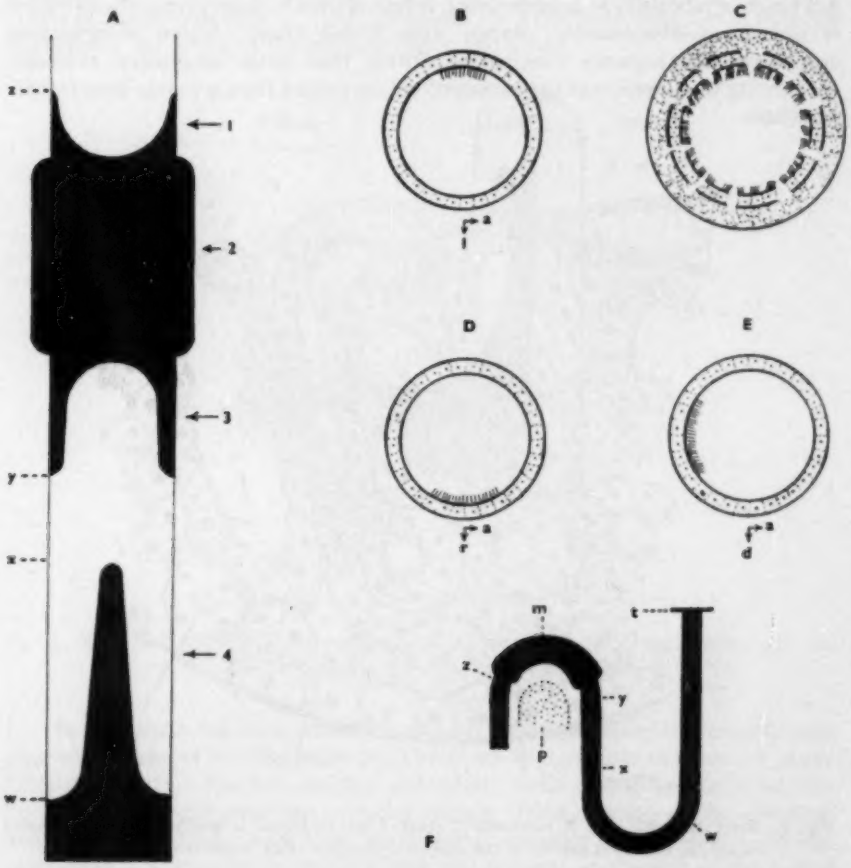

Fig. 2—Rana ribidunda. A diagram of the ciliation pattern of the larval fore-gut. A—ventral view of the fore-gut opened along the morphologically ventral midline with the cut edges reflected laterally. Ciliation tracts are shown in black. B, C, D and E—diagrammatic transverse sections through, respectively, levels 1, 2, 3 and 4 of 2A. F—lateral view of fore-gut showing the topographic relationships of the areas and levels figures in 2A. a, d, l and r, respectively, indicate the definitive relationships of the gut regions to the antero-posterior, dorso-ventral and lateral axes of the larva. (Key to lettering, p. 282).

by peristalsis (Fig. 3). It cannot be insignificant that in *Philautus gryllus* (*vide infra*) where cilia are completely absent from the *manicotto*, the latter is enclosed in a thick, muscular capsule.

Excepting the *manicotto*, the fore-gut tube consists simply of the epithelial layer successively enclosed in a moderately developed sheath of circular muscle and a very thin serosa. The *manicotto* differs from this general pattern, firstly, in the interpolation of a glandular layer between the epithelium and the muscle coat. These gland cells are cuboid in shape and possess a spherical nucleus situated basi-centrally. Their cytoplasm, which is granular and does not react significantly to mucus stains, is best shown in Azan preparations where it appears predominantly orange with bluish tinge. These observations emphasize Barrington's conclusions (1946) that little conclusive evidence concerning the function of the *manicotto* can be gained from a purely histological approach.

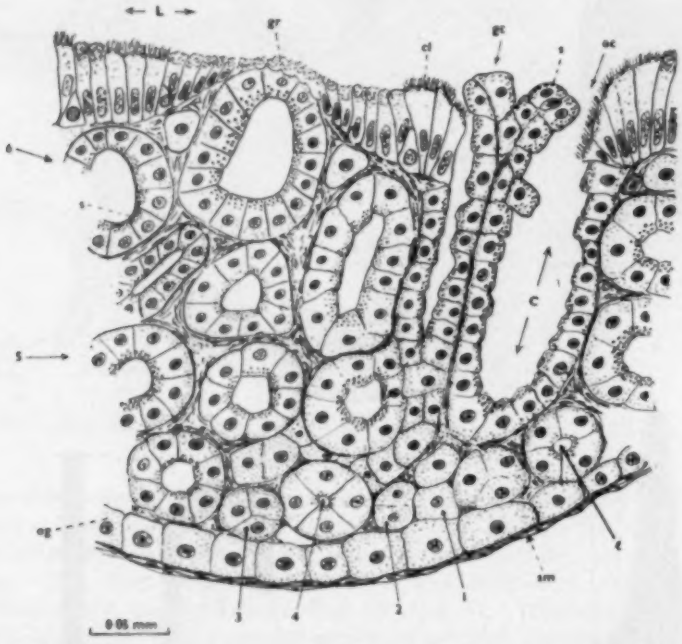

Fig. 3—*Rana ridibunda*. T. S. *manicotto* of stage V larva (Taylor & Kollros). 1–6, successive stages in the development of the *manicotto* glands. (Key to lettering, p. 282).

The gland typically consists of long cords arranged in a complex pattern of branching crypts which communicate with the gut lumen, not by a definite duct system, but through irregular gaps in the epithelium (Fig. 3 ; Pl. 1, fig. 2). Gland secretion is usually discharged into the crypts by rupturing of the gland cells. The latter then recover and recharge. Occasionally, particularly where the crypt elements project through the epithelial gaps, batches of gland

cells are budded of entire and discharged directly into the gut lumen (Pl. 1, fig. 2). Interpolated between the muscle coat and the *manicotto* is a fine network of blood capillaries which extend mesially into the connective tissue between the glandular cords (Pl. 1, fig. 2).

THE DEVELOPMENT OF THE *MANICOTTO* IN *RANA RIDIBUNDA*

Barrington (1946) believes that the glandular elements of the *manicotto* are formed developmentally as branched, tubular outgrowths of the epithelium and that this " may perhaps mean that the tubule cells are less fully developed forms of the surface cells ". This view does not agree with the present findings concerning the origin of the gland in *Rana ridibunda*.

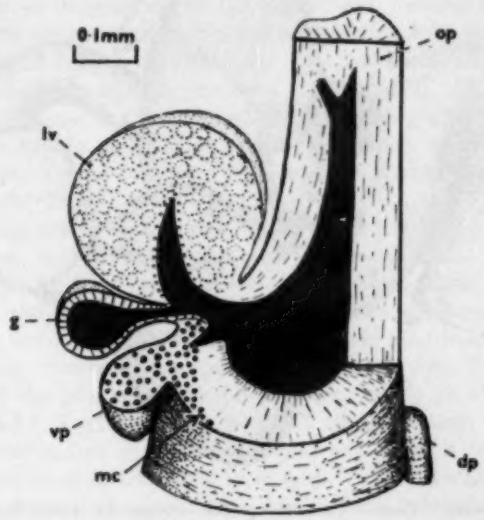

Fig. 4—*Rana ridibunda*. Fore-gut reconstruction of stage 22 larva (Rugh, 1951). The left anterior half of the gut tube has been removed. (Key to lettering, p. 282).

The *manicotto* begins its development at the stage when the oesophageal plug is still present and the pulmonary buds are beginning to cavitate (÷ stage 22, Rugh 1951). The two ventral pancreatic rudiments grow out from the floor of the fore-gut near the posterior margin of the opening of the bile duct and, as they enlarge, they fuse immediately anterior to the duct. At the same time a connection is retained with the fore-gut at the site of the initial proliferation. Along this bridge cells migrate back from the anlage to the gut, (Fig. 4 ; Pl. 1, figs. 3 and 4) and come to lie immediately beneath the epithelium which, at this stage, is many layered and still heavily loaded with yolk. The invading cells completely enclose the gut epithelium and extend anteriorly from their site of origin to form a discrete, sub-epithelial sleeve which constitutes the *manicotto* primordium (Fig. 5).

The *manicotto*/pancreatic bridge persists until stage 25 (Rugh, 1951) when it atrophies. Some species, however, e.g. *Rana macrodactyla*, *Heleophryne rosei* etc. (*vide infra*), retain the connection until the later stages of metamorphosis when, together with the *manicotto* and most of the larval pancreatic tissue, it degenerates.

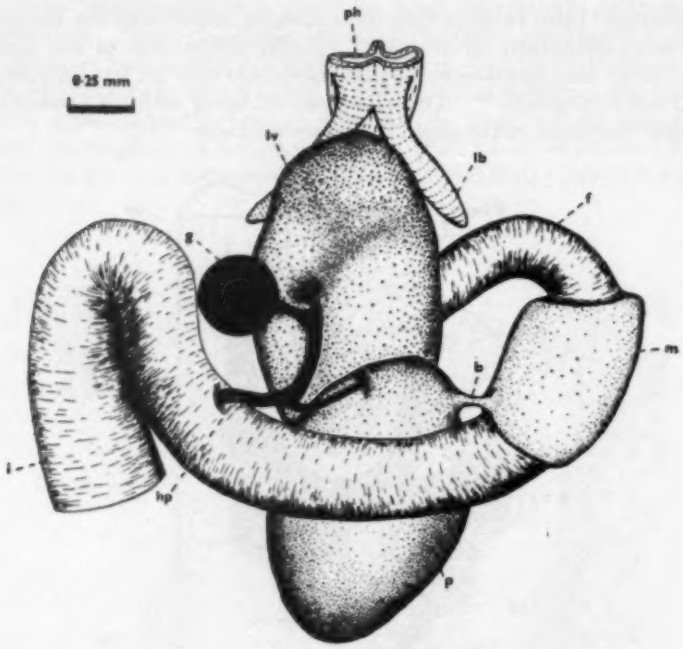

Fig. 5—*Rana ridibunda*. Ventral view of fore-gut of stage 25 larva (Rugh, 1951). (Key to lettering, p. 282).

Mitoses are numerous in the outer marginal layer of the *manicotto* and it is here that the initiation of the gland rudiments occurs, cell division in the main body of the *manicotto* being concerned with the maturation of existing elements rather than the initiation of fresh primordia. The gland rudiments divide to form a solid sphere of cells devoid of a lumen and without any evident secretory granules (Fig. 3). This stage is followed by one where the number of cells in the sphere has increased to its maximum (÷8–10 in transverse section), secretory granules are evident in the cytoplasm and the gland sphere has a lumen formed by dissolution of some of its central elements (Fig. 3). The gland spheres, immediately beneath the epithelium, press against the latter with a resulting stretching and thinning of the overlying epithelial cells which, together with the immediately subjacent cells of the sphere, rupture and release the gland secretion directly into the gut lumen (Pl. 5, fig. 21). It is noticeable that these epithelial breaks occur at points

where the overlying epithelium is glandular. This may be because the glandular elements of the *manicotto* lining represent areas of weakness which facilitate the breakthrough of the expanding spheres. Whatever the reason the result is that few, if any, ciliated elements are sacrificed in the process of rupturing and the epithelial borders of the rupture points are generally bordered by ciliated epithelium—a factor which, as has already been indicated, is of no little importance in maintaining flow through the *manicotto* and countering stagnation at the breakthrough sites where eddying is particularly liable to occur. It is noticeable also that, with the discharge of the gland and the resulting release of local pressure on the overlying epithelium, the cells of the latter are passively pulled into the neck of the gland opening (Fig. 3 ; Pl. 1, fig. 1), an appearance which, taken in isolation, might suggest that the gland elements develop as outgrowths of the epithelium. Gland spheres situated deeper in the *manicotto* invade and rupture surrounding spheres. The spherical identity of the gland units is thus gradually lost and the much-branched crypt system of the definitive organ is produced (Pl. 1, fig. 2). The complexity of the crypt pattern seems to be a characteristic of the genus concerned : it is not nearly so complex in ranid as in microhylid species, where the epithelium is so perforated as to cover less than fifty per cent of the lining surface in the definitive *manicotto* (Pl. 2, fig. 5).

Tadpole feeding ceases immediately prior to the loss of the larval jaws and direct observation, coupled with analyses of gut contents, shows that the fasting period lasts for from seven to ten days. About three days before the onset of fasting, radical degenerative and regenerative changes begin during which the entire larval gut epithelium and digestive glands are discarded and replaced by freshly constituted elements, which cope with the very different feeding habits of the post-metamorphic anuran. Early in this degenerative stage (≒ stage XXI, Taylor, & Kollros 1946), the cytoplasm of the *manicotto* glands becomes vacuolated and finely granular and stains a deep straw colour with the iron haematoxylin Orange G technique. The cell walls disappear and batches of gland elements become associated to form syncytial clumps (Pl. 2, fig. 6 ; Pl. 3, fig. 8). Cytoplasms of neighbouring clumps coalesce and the compound mass discharges into the gut lumen, via the epithelial gaps, carrying with it local concentrations of gland nuclei. The latter are frequently enormously swollen and abnormal and, even when obviously just about to be discharged into the gut lumen, often show aberrant, giant mitotic figures (Pl. 2, fig. 7). By progression of this degeneration-discharge process the glandular mass of the entire *manicotto* is finally discharged into the lumen. There is no evidence that any of the elements persist and dedifferentiate to form the glands of the definitive stomach as some authors have suggested (Kaywin, 1936 ; Barrington 1946).

These degenerative changes in the *manicotto* glands are accompanied by changes in the fore-gut epithelium, also. Here degeneration and reconstitution proceed *pari passu* in an antero-posterior direction. By stage XXII (Taylor & Kollros, 1946) the columnar elements of the epithelium are syncytial and contain numerous black, brown and yellow pigment masses together with many vacuoles. Cilia have almost completely disappeared and, for the first time,

Table 1

Family	Genus	Species	No. of specimens examined	*Manicotto*
Discoglossidae	*Alytes*	*obstetricans*	16 (47–56)	+(G)
	Bombinator	*igneus*	2 (48)	−(G)
		pachypus	1 (46)	−(G)
	Discoglossus	*pictus*	19 (43–58)	+(G)
Pipidae	*Xenopus*	*clivi*	2 (54)	+(F)
		laevis	85 (25–66)	+(F)
		tropicalis	16 (49–60)	+(F)
Pelobatidae	*Megophrys*	*hasselti*	21 (48–59)	−(G)
		major	16 (49–59)	−(F)
		pelodytoides	9 (48–56)	−(F)
Bufonidae	*Bufo*	*bufo*	56 (25–66)	−(G)
		calamita	7 (48–59)	−(G)
		latasti	4 (49)	−(G)
		melanosticus	12 (48–56)	−(G)
		parvus	14 (48–61)	−(G)
		penangensis	3 (49)	−(G)
Leptodactylidae	*Calyptocephalus*	*gayi*	2 (48)	+(G)
	Ceratophrys	*americana*	1 (49)	−(G)
		laevis	1 (49)	+(FE)(C)
	Heleioporus	*centralis*	1 (50)	+(G)
		eyrei	1 (49)	+(G)
	Heleophryne	*rosei*	1 (49)	+(G)
	Pleurodema	*bibroni*	4 (51)	+(G)
		cinerea	1 (49)	+(G)
		marmorata	3 (49)	+(G)
	Pseudophryne	*bibroni*	1 (52)	+(G)
		semimarmorata	1 (51)	+(G)
	Telmatobius	*marmoratus*	1 (51)	+(G)
Hylidae	*Hyla*	*dominicensis*	11 (48–59)	+(G)
		everetti	2 (49)	+(G)
	Gastrotheca	*marsupiatum*	4 (50)	+(P)
		testudineum	3 (53)	+(P)
	Nyctimystes	*montana*	1 (49)	+(G)
Ranidae	*Cacosternum*	*boettgeri*	34 (46–64)	+(G)
	Ooeidozyga	*laevis*	15 (49–65)	+(C)
		lima	21 (49–60)	+(C)
	Rana	*afghana*	2(53)	+(G)
		alticola	12 (48–65)	+(G)
		cancrivora	19 (48–62)	−(G)
		catesbiana	16 (48–64)	+(G)
		chalconota	2 (51)	+(G)
		cyanophlyctis	2 (49)	+(C)
	Rana	*erythraea*	3 (54)	+(G)
		esculenta	15 (48–65)	+(G)
		kuhli	4 (53)	−(G)
		lateralis	7 (48–59)	+(G)
		limnocharis	16 (45–60)	−(G)
		luctuosa	5 (49)	−(G)
		macrodactyla	16 (46–56)	+(G)
		macrodon	4 (52)	+(G)
		macrognathus	7 (52–60)	−(G)

Table I—*continued*

Family	Genus	Species	No. of specimens examined	*Manicotto*
		nigrovittata	23 (44–65)	+(G)
		plicatella	3 (51)	+(G)
		ridibunda	92 (32–66)	+(G)
		rugulosa	11 (48–60)	+(C)
		temporaria	73 (35–66)	+(G)
		tigrina	9 (49–60)	+(C)
		whiteheadi	2 (49–56)	+(G)
	Staurois	*larutensis*	17 (49–56)	−(G)
Rhacophoridae	*Megalixalus*	*seychellensis*	2 (52)	+(G)
	Nyctibatrachus	*major*	2 (49)	−(G)
	Philautus	*gryllus*	21 (46–66)	+(FD)(G)
		vittatus	7 (49–59)	+(G)
	Rappia	*betsileo*	2 (52)	+(G)
	Rhacophorus	*bimaculatus*	6 (48–56)	+(G)
		leucomystax	15 (48–65)	+(G)
Microhylidae	*Calluella*	*guttulata*	27 (45–66)	+(F)
	Chaperina	*fusca*	1 (49)	+(F)
	Glyphoglossus	*molossus*	1 (54)	+(F)
	Hypopachus	*aguae*	4 (49–63)	+(F)
	Microhyla	*achatina*	3 (53)	+(F)
		berdmorei	4 (49)	+(F)
		butleri	16 (45–66)	+(F)
		heymonsi	5 (52–60)	+(F)
		rubra	23 (45–66)	+(F)

Abbreviations : C. carnivorous feeding habits ; F. filter feeder ; FE. stomach-like expansion of fore-gut ; FD. fore-gut with well defined diverticulum ; G. indiscriminate feeder.
The bracketted figures indicate the range of developmental stages examined and are based on comparisons with the Normal Table for *Xenopus laevis* (Nieuwkoop & Faber, 1956). The staging recorded in subsequent tables, also, are based on the same scale.

the degenerating " round cells " (Duesberg, 1906 ; Bowers, 1909) are apparent (Pl. 3, fig. 9). These are discrete syncytial nests loaded with dense, yellow secretory granules and obviously in an advanced stage of degeneration. In contrast to the above degenerating epithelial elements, the basal cells, which hitherto have been inconspicuous and few in number, are actively dividing to produce regional, syncytial bands extending discontinuously beneath the degenerating columnar epithelium of the larva. When discharge of the regressing *manicotto* glands is complete, these syncytial bands merge edge to edge and completely enclose the larval epithelium which, now, completes its degeneration and is sloughed into the gut lumen. The syncytial bands develop cell walls and give rise to the definitive, post-metamorphic epithelium. At the same time they produce outgrowths which extend into the surrounding sub-mucosa to form the glands of the adult fore-gut (stomach and oesophagus). Concurrently, proliferative changes occur to produce the muscularis mucosa, the sub-mucosa, the circular muscle layer and the serosa (Pl. 3, figs. 10 and 11) typical of the newly metamorphosed frog. These changes are complete by stage XXV (Taylor & Kollros, 1946).

The above account of the epithelial changes agrees closely with that of Duesberg (1906) for *Rana fusca* and Bowers (1909) for *Bufo lentigenosus* and is applicable to all regions of the fore-gut. It agrees, also, with events in all the other species whose development was followed during the present investigation (Table 1). Similarly, all the glands of definitive fore-gut (gastric and oesophageal) in these species arise as new developments from the reconstituted epithelium. Concerning the glands of the post-metamorphic fore-gut, it is well known that, in the toad, the ciliated oesophagus is gland-free (Langley, 1881 ; Machan, 1935) whilst, in the frog, there occur the so-called " oesophageal glands " (Machan, 1935). There is also evidence that these oesophageal glands secrete pepsin but not acid (Langley, 1881 ; Friedman, 1937). During the course of the present investigation different species were examined for the basiphil oesophageal glands (Table 2). Clearly no correlation exists between the presence of the glands in the adult and of the *manicotto* in the larva (Table 1). It is interesting to note, nevertheless, that their distribution seems restricted to ranoid Anura and that they occur in both *Dendrobates* and *Phyllobates*—genera whose affinities to the ranoids have recently been suggested from other quite different criteria (Griffiths, 1959).

THE FORE-GUT IN LARVAE OF OTHER ANURAN SPECIES

Tadpoles from nine families representing thirty genera and seventy-four species were examined. All species where the organ is recorded as absent were investigated both by dissection and as serial sections, as were most of the forms for whom positive results are shown. A few of the latter, however, were studied by dissection only. Data concerning the species examined and the results obtained are collated in Table 1.

In the species without a *manicotto* the general form and histology of the fore-gut agrees closely with that described by Barrington (1946) for *Bufo bufo*. The only exception encountered was in the tadpole of *Bufo calamita* where the transition from the fore- to the mid-gut is sharply demarcated by a sphincter constriction. The transition is further emphasized by the presence, immediately posterior to the constriction, of a mid-gut diverticulum into which the hepato-pancreatic duct opens.

The *manicotto*, in nearly all cases, agrees almost exactly in topographical and histological pattern with that of *Rana ridibunda*. Some variations do, however, occur. Firstly, in all the microhylid species examined the complexity of the crypt pattern and the extensiveness of the crypt spaces are far greater than in any ranid form ; and, probably correlated with this type of crypt development, the proportion of epithelium which persists in the definitive *manicotto* is significantly less than in the tadpoles of any other family (Pl. 2, fig. 5). This high degree of epithelial breakdown makes for greater intimacy between the glands themselves and the food passing through the *manicotto* and, clearly, is possible only in larvae whose feeding habits preclude the entry of abrasive particles into the gut. The budding off of entire glandular cords seems to play a far greater role in *manicotto* function in microhylid larvae than in those of other families and is evidently correlated with the high degree of epithelial breakdown in these forms. And the extreme microphagy of the

Table 2

Family	Genus	Species	Oesophageal glands
Discoglossidae	*Alytes*	*obstetricans* (1)	−(S)
	Bombinator	*igneus* (1)	−(S)
	Discoglossus	*pictus* (6)	−(S)
Pipidae	*Hymenochirus*	*boettgeri* (1)	−(S)
	Xenopus	*laevis* (12)	−(S)
		tropicalis (3)	−(S)
Pelobatidae	*Megophrys*	*hasselti* (1)	−(S)
		major (7)	−(S)
Bufonidae	*Bufo*	*bufo* (5)	−(S)
		calamita (2)	−(S)
		marinus (1)	−(S)
		viridis (3)	−(S)
	Cacophryne	*borbonica* (4)	−(S)
	Dendrophryniscus	*stelzneri* (2)	−(S)
	Nectophrynoides	*vivipara* (3)	−(S)
	Oreophrynella	*quelchi* (2)	−(S)
	Pedostibes	*guentheri* (3)	−(S)
	Rhinoderma	*darwini* (2)	−(S)
	Sminthillus	*limbatus* (3)	−(S)
Atelopodidae	*Atelopus*	*zeteki* (1)	−(S)
	Brachycephalus	*ephippium* (2)	−(S)
Leptodactylidae	*Eleutherodactylus*	*luteus* (1)	−(S)
	Leptodactylus	*pragnathus* (1)	−(S)
Hylidae	*Hyla*	*dominicensis* (2)	−(S)
Ranidae	*Cacosternum*	*boettgeri* (2)	+(G)
	Dendrobates	*tinctorius* (2)	+(G)
	Phyllobates	*femoralis* (1)	+(G)
		trinitatus (1)	+(G)
	Rana	*esculenta* (5)	+(G)
		ridibunda (2)	+(G)
		rugulosa (1)	+(G)
		temporaria (5)	+(G)
	Staurois	*larutensis* (2)	+(G)
Rhacophoridae	*Rhacophorus*	*bimaculatus* (2)	+(G)
		leucomystax (1)	+(G)

Abbreviations : S. and G., respectively, indicate an abrupt or a gradual histological transition from oesophagus to stomach.

microhylid tadpole may not be unrelated to these modifications. Concerning this latter point, however, it should be recalled that the xenopid larvae are equally microphagous and yet their *manicotto* structure is essentially of the ranid type. A second group of species which deviates from the *Rana ridibunda* type of *manicotto* includes *Rana macrodactyla*, *Heleophryne rosei* and all the xenopid species which were examined (Table 1). These forms retain the *manicotto*/pancreatic bridge until late metamorphosis when gut degeneration and reconstitution is taking place (Fig. 6 ; Pl. 4, fig. 15). Thirdly, the *manicottos* of *Ceratophrys laevis*, *Ooeidozyga laevis* and *O. lima* differ from the generalized pattern in (*a*) being enclosed in a thick muscular coat ; (*b*) possessing no cilia and (*c*) having the number of epithelial perforations greatly reduced.

These features are clearly linked with the exclusively carnivorous habits of these tadpoles. Gut analysis shows that they feed chiefly on insects (see also Smith, 1916) and it seems likely that, in them, the *manicotto* functions, in part, as a triturating chamber.

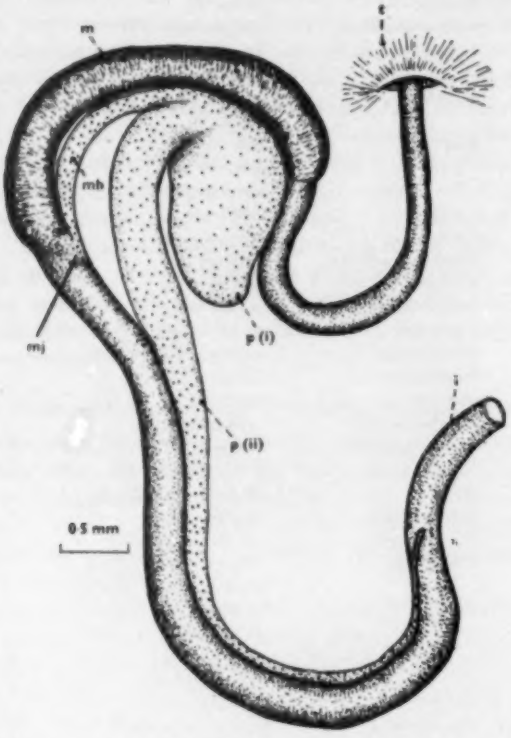

Fig. 6—*Heleophryne rosei*. Fore-gut of stage VI larva (Taylor & Kollros). Ventral view. (Key to lettering, p. 282).

The above species differ from the typically ranid condition only in degree : in retaining throughout larval life features which are present in *Rana ridibunda* in early developmental stages only (*manicotto*/pancreatic bridge) ; in elaborating characters which are only moderately developed in the more typical *manicotto* (glandular crypts and muscle coat), or in suppressing the development of such characters (cilia and epithelial perforations). Two other species, *Alytes obstetricans* and *Philautus gryllus*, have a *manicotto* whose pattern is completely different from the generalized ranid type and in which other regions of the fore-gut have also become modified.

The fore-gut of the *Alytes* tadpole is sharply demarcated from the mid-gut by a diverticulum situated at the beginning of the latter (Figs. 7–9). As in *Bufo calamita*, this diverticulum receives the hepato-pancreatic duct. Gross dissection gives no indication that a *manicotto* is present but the fore-gut is

characterized by a number of longitudinal grooves on the anti-pancreatic (morphologically ventral) side of the proximal limb of the second gut loop (Fig. 7). Histological examination shows that typical *manicotto* tissue extends from the apex of the first gut loop and continues for half the length of the distal limb leading to the second loop (Fig. 9A-F). The condition is atypical in that the gland does not encircle the gut, as in *Rana ridibunda*, but is limited to the pancreatic (morphologically dorsal) half of the gut tube (Fig. 9 D & E). This suggests that the *manicotto* in this species may be derived from the dorsal pancreatic rudiment only. But developmental material was not available to examine this point.

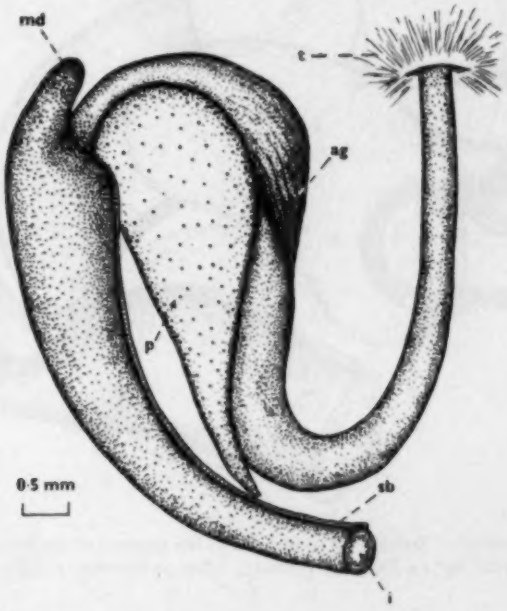

Fig. 7—*Alytes obstetricans*. Ventral view of fore-gut of stage IX larva (Taylor & Kollros). (Key to lettering, p. 282).

Immediately behind the anterior tip of the manicotto the fore-gut wall, directly opposite the gland, involutes into the gut lumen forming a bifid tongue with a muscular core (Fig. 9 A-C ; Pl. 4, fig. 14). Posteriorly the involution unfolds and its lateral margins open to form two pronounced grooves with shallower, subsidiary furrows between them. The marginal grooves become progressively shallower until, at the apex of the second gut loop, they are distinguishable from the subsidiary furrows only by their lateral positions (Fig. 9B). This condition persists up to the end of the fore-gut. Three well defined concentrations of circular muscle are present between the oesophagus and the intestine : (*a*) a strong sphincter enclosing the apex of the second gut loop (*N*-Fig. 9F) ; (*b*) a weaker band situated immediately anterior to the

manicotto (*O*-Fig. 9 F) ; (*c*) a moderately developed ring enclosing the opening of the fore-gut into the mid-gut diverticulum (*sp*-Fig. 9 F). In addition to the above, a hemisphincter situated on the same side of the gut as the *manicotto*, extends from immediately behind the posterior border of the latter up to the apex of the second gut loop (Fig. 9 B). The functional significance of these muscle aggregations will be discussed later.

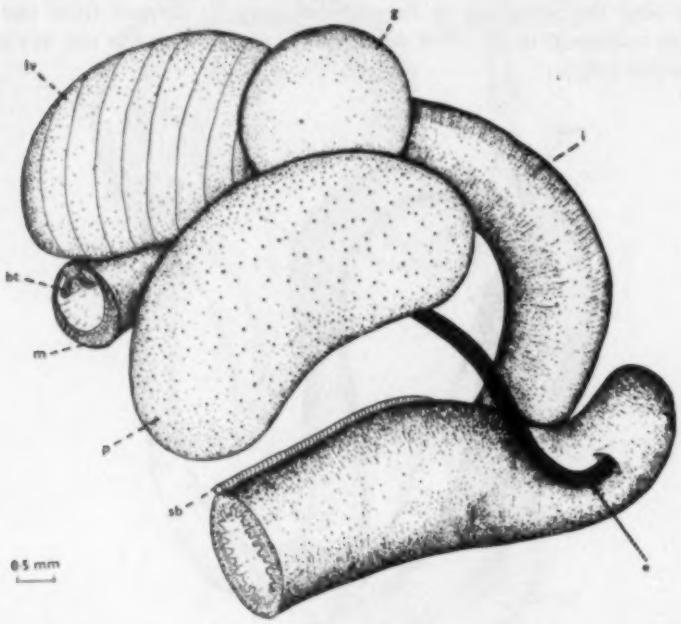

Fig. 8—*Alytes obstetricans*. Dorsal view of fore-gut at the junction of the fore-gut and intestine. (Stage IX larvae, Taylor & Kollros). (Key to lettering, p. 282).

Cytologically, the *manicotto* elements of *Alytes* do not differ significantly from those of *Rana ridibunda*. The gland epithelium, however, is much more richly supplied with goblet cells. The ciliation pattern, also, is different. Cilia extend continuously from the oesophagus up to the apex of the first gut loop (Fig. 9 A and F) where they are absent from the short region occupied by the first sphincter muscle. This is followed by a narrow ciliated band which extends completely round the gut up to the anterior tip of the *manicotto* where it becomes restricted to a tract occupying about one third of the gut circumference and situated in the anti-pancreatic (morphologically ventral) side of the fore-gut. The muscular tongue and the fore-gut grooves, noted above, are included in the tract and the ciliary beat is lateral on the former and postero-anterior on the latter. Immediately posterior to the main sphincter, the lateral margins of the tract converge and the fore-gut epithelium retains this completely ciliated pattern until it enters the mid-gut (Fig. 9 A).

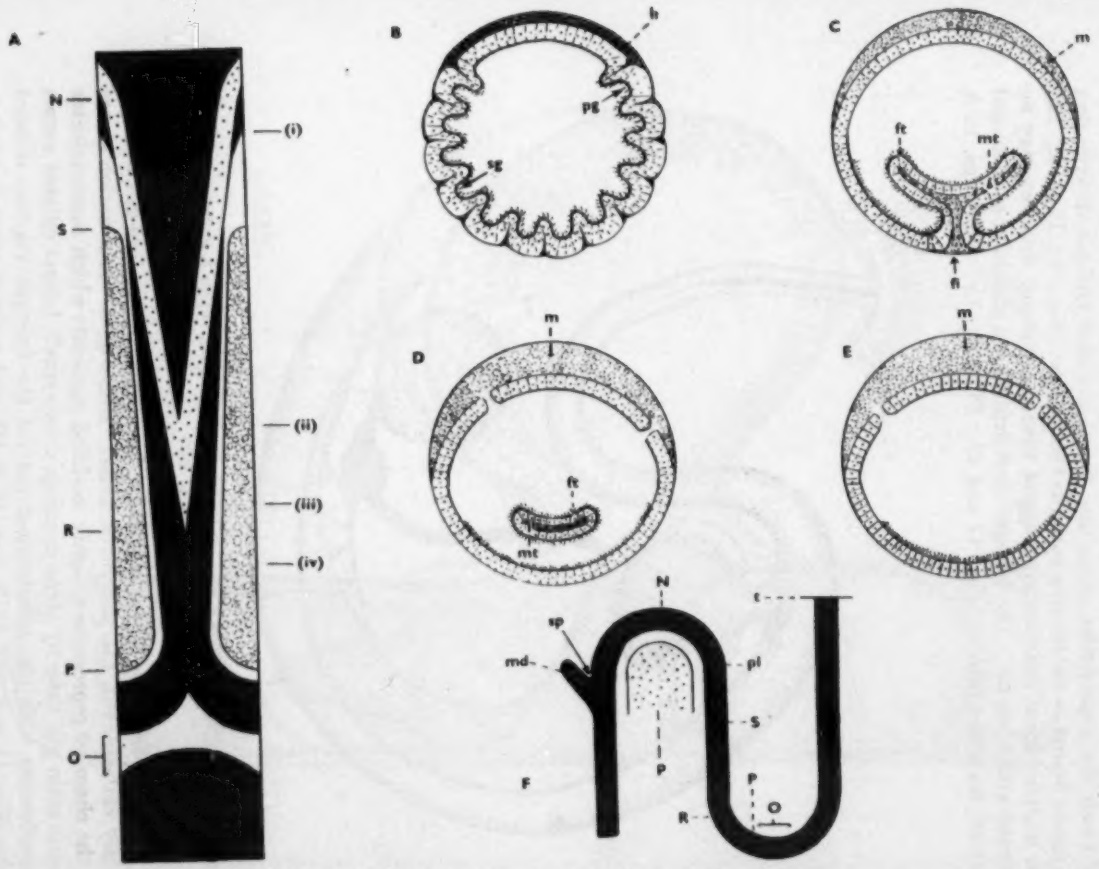

Fig. 9—*Alytes obstetricans*. Ciliation diagram of larval fore-gut.

A—dorsal view of gut opened along the morphologically dorsal midline with the cut edges reflected laterally. Ciliation tracts shown in black ; fore-gut tongue and primary grooves in coarse stipple ; *manicotto* in stippled circles. B, C, D and E—diagrammatic transverse sections through, respectively, levels (i), (ii), (iii) and (v) of 9A. F—lateral view of fore-gut showing the topographic relationships of the different areas and levels figured in 9A. (Key to lettering, p. 282).

The alimentary canal of the *Philautus gryllus* tadpole (Figs. 10–12) is unique among all anuran tadpole guts hitherto described in possessing a mid as well as a fore-gut diverticulum. Both diverticula are roughly spherical in outline. At its distal end the hepatopancreatic duct widens into a reservoir from which the main outlet opens into the thin-walled mid-gut diverticulun with minor branches discharging towards the fore-gut (Fig. 10 ; Pl. 4, fig. 12). These morphological relationships suggest that the mid-gut chamber may be concerned with lipolysis. The fore-gut diverticulum is a glandular sac enclosed in a thick, muscular capsule (Figs. 11 and 12 ; Pl. 4, fig. 13 ; Pl. 5, figs. 16 &

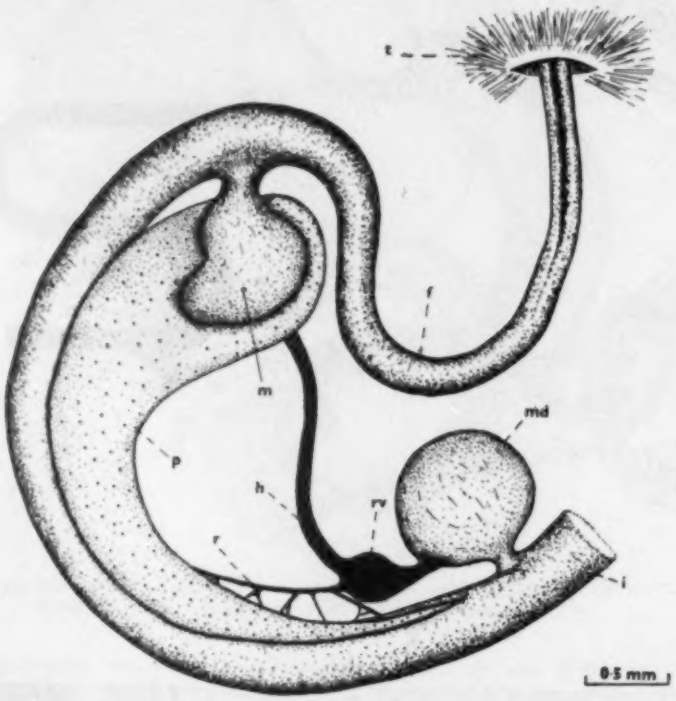

Fig. 10—*Philautus gryllus*. Ventral view of fore-gut (Stage V larva, Taylor & Kollros). (Key to lettering, p. 282).

17). It arises from the morphologically dorsal side of the fore-gut and lies partially embedded in the pancreatic mass. Histological examination shows that the chamber represents a highly modified *manicotto* which communicates with the main gut tube by three openings : two small, lateral ciliated canals communicating with the anteriormost part of the fore-gut via two ciliated grooves (Fig. 11 and 12 ; Pl. 5, figs. 16 & 17), and a single ventral opening. The latter is covered, trapdoorwise, by a posteriorly directed triangular flap. This flap can open ventrally into the lumen of the gut but is prevented from opening dorsalwards, into the chamber, by a thick, connective tissue flange

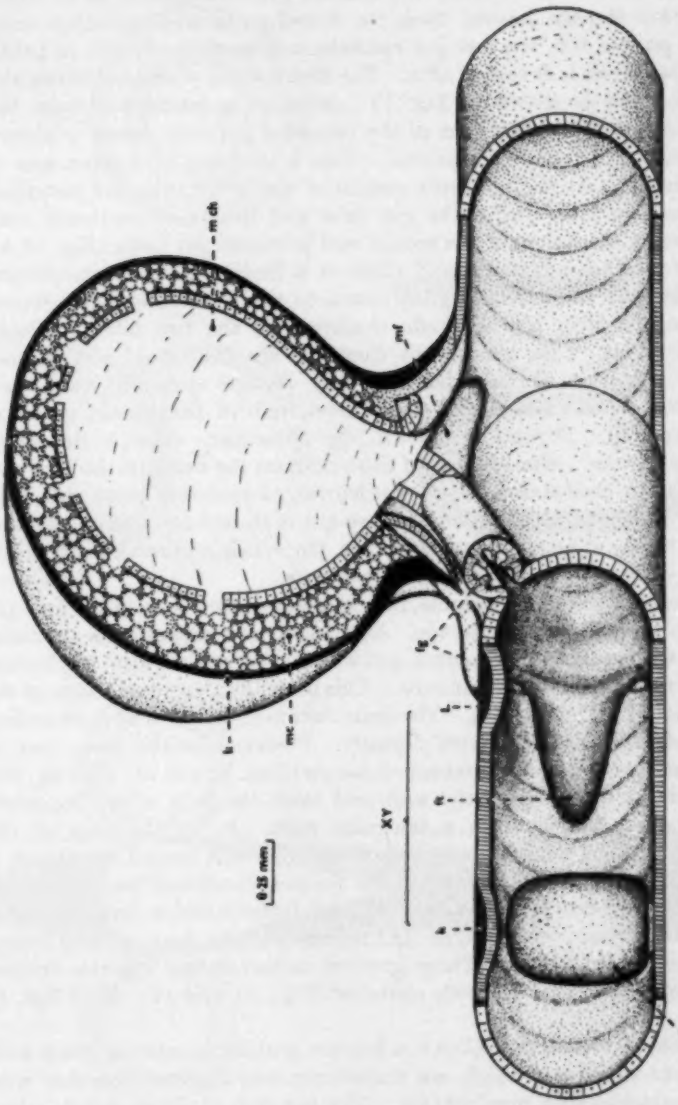

Fig. 11—*Philautus gryllus*. Lateral view of fore-gut. Half of the *manicotto* capsule and parts of the fore-gut tube have been removed. (Stage V larva, Taylor & Kollros). (Key to lettering p. 282).

situated on the posterior margin of the ventral opening into the *manicotto* (Fig. 11). The trapdoor thus permits egress from but prevents entry into the diverticulum.

The disposition of the *manicotto* in *Philautus gryllus* suggests, as in *Alytes obstetricans*, that it may develop from the dorsal pancreatic rudiment only. In *Philautus gryllus*, too, the fore-gut epithelium is particularly rich in goblet cells and the *manicotto* is devoid of cilia. The distribution of cilia in the remainder of the fore-gut is shown in Fig. 12 : ciliation is continuous from the oesophagus to just before the apex of the proximal gut loop, where a narrow band of epithelium is entirely cilia-free. This is followed by a short area of complete ciliation. At the posterior margin of the latter, cilia are restricted to the ventro-lateral surface of the gut tube and this tract continues until half-way between the apices of the second and proximal gut loops (Fig. 12 A). At this point, the tract diverges and ciliation is limited to two dorso-lateral tracts which include, and extend slightly lateral to, two dorso-lateral gut grooves that communicate with the *manicotto* chamber via the two lateral ciliated canals noted above. Cilia are absent from the chamber itself and, almost immediately posterior to the chamber, they become restricted to a tract extending for a short distance into the distal limb of the second gut loop (Fig. 12 A and B). Beyond this point, the alimentary canal is devoid of cilia up to the rectum. The absence of ciliation from the bends of the fore-gut, in both *Philautus gryllus* and *Alytes obstetricans*, is probably correlated with the relatively heavier musculation of the fore-gut in these tadpoles and suggests that in their case, unlike most anuran larvae, peristalsis and not ciliary action is the primary agent for moving fore-gut contents.

As in the case of the alytid tadpole, the extra-*manicotto* region of the fore-gut is modified in the philautid larva, too. About half-way between the proximal gut loop and the *manicotto*, the ventral gut wall is involuted to form a spherical plug with a solid, connective tissue core. This plug fills about two thirds of the gut lumen (Fig. 11 ; Pl. 5, fig. 18). The musculature of the gut wall surrounding the plug is significantly thickened dorsally. Posterior to the plug, just in front of the *manicotto*, a second involution occurs (Figs. 11 and 12, Pl. 6 fig. 20). This lies laterally, on the left-hand wall, and takes the form of an elongated, forwardly-directed tongue with a muscular core. As in the case of the anterior plug, the gut wall musculature surrounding this second involution is also thickened. Between the plug and the tongue, the dorsal wall of the gut is corrugated into a number of shallow, ciliated furrows which converge, level with the posterior end of the tongue, and continue as the deep, ciliated dorso-lateral grooves noted above. These grooves communicate, via the ventro-lateral openings, with the *manicotto* chamber (Figs. 11 and 12 ; Pl. 5 figs. 16 and 17).

The tadpole of *Philautus gryllus* is a bottom grubber inhabiting pools with highly siliceous substrates which are indiscriminately ingested together with any organic material they may contain. The function of the modified, extra-*manicotto* portion of the fore-gut is clearly to separate the food particles from the larger inorganic substrate elements and to ensure that only the former pass into the *manicotto* chamber. The efficiency of the system is evidenced

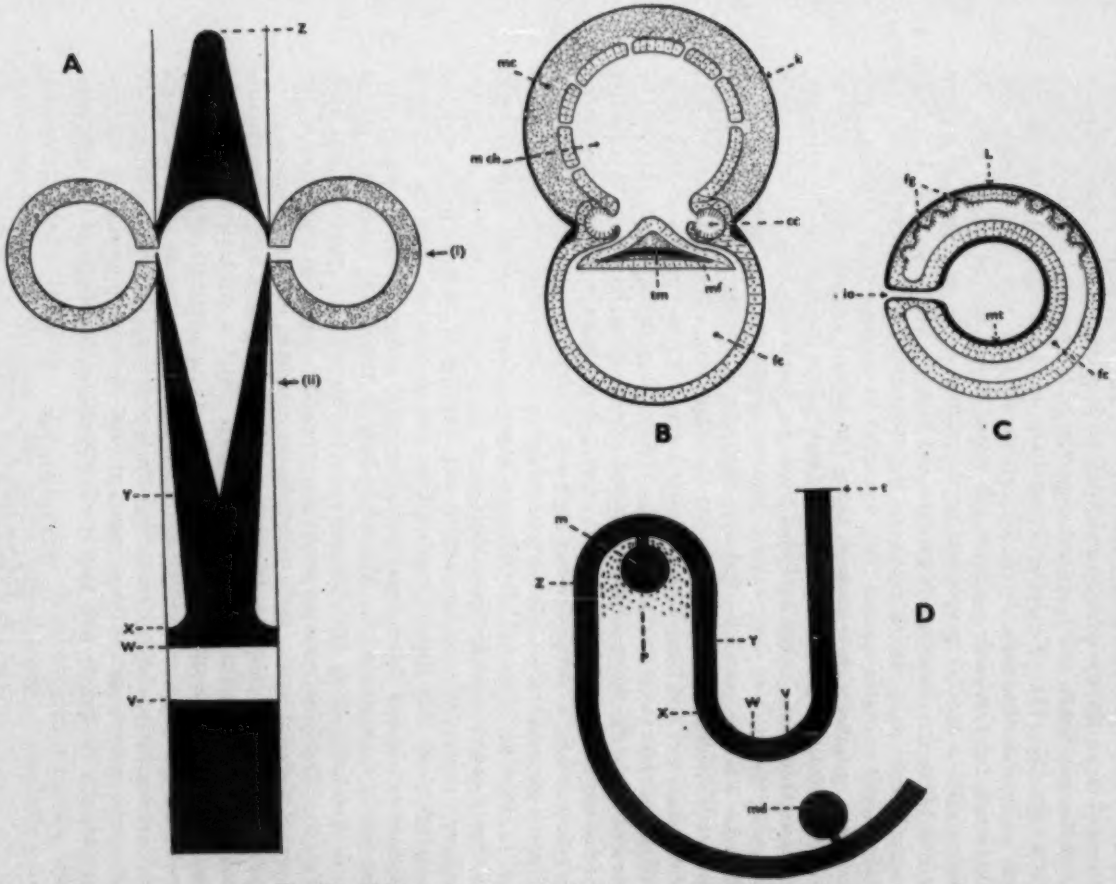

Fig. 12—*Philautus gryllus*. Ciliation diagram of larval fore-gut.
A—dorsal view of fore-gut opened along the morphologically dorsal midline with the cut edges reflected laterally. Ciliation tracts shown in black and *manicotto* tissue in stipple. B and C—diagrammatic transverse sections through, respectively, levels (i) and (ii) of 12A. D—lateral view showing topographic relationships of the different areas and levels indicated in 12A. (Key to lettering, p. 282).

by the fact that none of the sand grains which pack the remainder of the alimentary canal are ever found in the fore-gut diverticulum. Consideration of the fore-gut structure suggests that the separation mechanism may function as follows. Substrate material, together with any food matter it may contain, is passed down the oesophagus and localized in section XY by constriction of sphincter muscles K and L around respectively, the fore-gut plug and the fore-gut tongue (Fig. 11). The latter, by sporadic contractions of its core musculature, aggitates the contents of XY whose particles will obey Stoke's law. The small, light, organic particles will settle slowly and be carried with the ciliary stream (probably enclosed in the mucus cords so typical of anuran larvae) along the dorso-lateral grooves and into the *manicotto* (Fig. 11). The larger, heavier substrate elements, on the other hand, settle more quickly and their weight militates against their transport in mucus cords. Further, their large size precludes their passage along the lateral grooves and, since these grooves constitute the only channels of entry into the fore-gut diverticulum, the inorganic particles are excluded from the *manicotto*, also. When muscle L is contracted, the dorso-lateral grooves constitute, not merely the only entrance into the *manicotto*, but also the only exit route, posteriorly, out of section XY. This means that during the aggitation/selection process in XY, when muscles K and L are both contracted, the only material to pass backwards will be that destined for the *manicotto*. The residuum (mainly inorganic) will be released only when the sphincters relax and, side-tracking the *manicotto*, will enter the mid-gut direct. Discharge of the *manicotto* contents is clearly not operated by ciliary action. The mechanism probably involves simultaneous contractions of (*a*) the transverse muscle in the triangular flap of the ventral trapdoor (Fig. 11 and 12; Pl. 5, fig. 17) and (*b*) the muscular capsule of the *manicotto* chamber itself.

The fore-gut modifications that occur in the alytid tadpole are, also, probably concerned with the sorting and selection of food particles, and the morphology of this region suggests that the mechanism involves localizing the material passed down the oesophagus in gut segment ON (Fig. 9), by constriction of the sphincters*. The ciliary beat in this region carries the mucus cords forwards, along the fore-gut grooves, towards the proximal gut loop (Fig. 9). Food particles are probably segregated from the remainder of the ingested material by the same principles as suggested for the philautid larva, the agitating mechanism, in this instance, being probably fore-gut peristalsis and contraction of the muscular involution. Such a mechanism can divide the contents of segment ON into two fractions : (*a*) corded food particles concentrated and continuously circulated proximally in the region of the *manicotto* and, consequently, most subject to the latter's secretions, (*b*) the heavier (possibly inorganic) constituents of the ingest packed distally, immediately anterior to sphincter N (Fig. 9), and thus least exposed to the effects of the *manicotto*.

EXPERIMENTAL

A. *Determination of enzymes*

Kunz (1924) recorded peptic activity in early post-metamorphic stages of *Rana pipiens* but found no evidence that the enzyme occurs in the larval

*Since this manuscript was submitted, living alytid tadpoles have become available and the food sorting mechanism, here proposed from purely morphological considerations, has been confirmed experimentally. The role of peristalsis in the movement of gut contents (suggested overleaf, p. 268) is also confirmed.

fore-gut of this species. Doljanski (1933), using homogenates of entire *Rana temporaria* tadpoles, concluded that pepsin is absent from these larvae, also, but that trypsin, dipeptidase and kathepsin are all active. Barrington (1946), investigating fore-guts of *Rana temporaria* and *Bufo bufo*, endorsed Kunz's conclusion that pepsin becomes detectable in Anura only after metamorphosis ; he recorded, further, that no fore-gut amylase occurs in premetamorphic stages. Barrington questioned the validity of Doljanski's results, at least so far as gut digestion is concerned, because her estimations were made on extracts of entire larvae and, also, because she failed to detect pepsin in early froglet stages. On the basis of his own results and those of Kunz (1924) Barrington, therefore, concluded that function of the *manicotto* is unlikely to be enzymic and, tentatively, he suggested that it might function as a supplementary mucus organ.

The morphological modifications of the *manicotto* and the fore-gut in *Alytes obstetricans* and, particularly, in *Philautus gryllus* seem far too elaborate to be explained in this way. In the latter species the filtering apparatus clearly ensures that only fine particles are carried into the fore-gut (*manicotto*) chamber. Barrington's suggestion that a mucus secreting device would have value " in animals where passage of so much rough and fragmented material has be to lubricated " is inapplicable, here, since the very material excluded by the chamber mechanism is the macro-particulate grit and sand which, according to the above argument, would most require lubrication. Further, it is not insignificant that an extensive *manicotto* is invariably present in free-swimming pipid and microhylid larvae, all of which are filter feeders and enclose their food in mucus cords prior to its entry into the fore-gut. In these forms no problem of " intestinal abrasion " can possibly exist. The embryonic origin of the organ and, in many cases, its persistent attachment to the pancreatic tissue, also suggest that its role may not be simply to facilitate the physical passage of ingested material through the alimentary tract. Moreover, Doljanski's work cannot be completely ignored for her results (at least for premetamorphic stages) are supported by those of Løvtrup (1955), who estimated proteolytic activity in larval homogenates of *Xenopus laevis*. In view of these points it seemed pertinent to re-examine the tadpole fore-gut for enzymic activity.

The material investigated consisted of *Bufo bufo*, *B. calamita*, *Rana ridibunda*, *R. temporaria* and *Xenopus laevis* tadpoles at stages extending from the early feeding larva to about eight weeks after the completion of metamorphosis. For the purposes of analysis the fore-gut was divided into three regions (Fig. 1) extending (*a*) from the transverse septum to the anterior border of the *manicotto* : (*b*) the entire length of the *manicotto* and (*c*) from immediately behind the *manicotto* up to (but not including) the hepato-pancreatic duct. Particular care was taken to avoid contamination with pancreatic tissue. In the case of the bufonid larvae, where no *manicotto* tissue occurs, extractions were made of the fore-gut as a whole.

The Gunzberg test for free hydrochloric acid was negative for all fore-gut regions in every species examined. Since the primary concern of this investigation is with the *manicotto*, pH values were estimated only for region (*b*), above.

Manicotto contents from twenty-five tadpoles (developmental range 1–X, Taylor & Kollros, 1946) were diluted with three drops of distilled water and the pH measured electrometrically. Five such estimations were made for each species and the pH range recorded extended from 7·4 to 7·9 with a mean of 7·8. These results preclude the possibility of peptic activity and thus confirm the conclusions of Kunz (1924) and Barrington (1946) who tested directly for the enzyme itself.

Extracts for enzyme analyses were prepared by treating finely minced fore-gut tissue with four times its weight of 25 per cent ethanol for three days at 20°C. Lipolytic activity was investigated by pipetting small drops of the extracts on the surface of a cooled, homogeneous emulsion of neutral fat in forty times its volume of agar-agar. The digestion plates were incubated at 38°C for one to three hours, together with control dishes in which 25 per cent ethanol was substituted for gut extract. At the end of the incubation period the surfaces of the agar plates were flooded with aqueous copper sulphate solution and left for ten minutes. They were then washed in distilled water. A few plates gave slight, blue-green deposits of copper soap suggesting lipase activity. A quantitative estimation of this enzyme (using the method of Willstätter, Waldschmidt-Leitz & Memmen), however, made it doubtful that it plays any significant role in larval digestion (Table 3).

Table 3—Lipase activity is expressed as mls. of ethanolic KOH required to neutralize the products of the hydrolysis of 0·25 g. of olive oil (saponification number 185) by the enzyme preparation. The values recorded are corrected against the titration values of the buffer and enzyme solutions.

Species	Stage	No. of specimens per batch	No. of batches	Mls. 0·1 N ethanolic KOH
Rana temporaria	50	7	4	0·01
	54	10	5	0·01
	56	9	5	0·03
	60	10	5	0·02
Xenopus laevis	52	8	5	0·03
	55	10	4	0·01
	59	6	2	0·04
	62	10	5	0·03

Further samples of the gut extracts were mixed with three times their volume of starch paste and incubated at 40°C. Portions of the digest-mixtures were withdrawn at periods varying from 30 minutes to 24 hours and each portion divided into two equal parts ; one of these was tested with iodine for undigested starch and the other by Benedict's test for reducing sugar. None of these tests indicated any degree of amylase activity. This accords with the conclusions of Barrington (1946).

Proteolysis was estimated by formol titration. 0·5 ml. of gut extract was added to 10 ml. of 8 per cent casein solution (Hammersten's), and, as a precaution against putrefaction, a few drops of toluene were added, routinely, to each experimental tube. A control containing boiled extract was also prepared and both blank and digest solutions were set to incubate at 38°C. A titration

standard was set up by adjusting 1 ml. of boiled extract to pH 8·5 with 0·05 N sodium hydroxide solution, using phenolphthalein as the indicator. All subsequent titrations in this experiment were made to match this end-point. At recorded intervals (Table 4 and 5) 1 ml. samples of digest and control solutions were withdrawn, one drop of 0·5 per cent ethanolic phenolphthalein added to each and the whole rapidly titrated with 0·1 N sodium hydroxide to match the titration standard. 0·25 ml. of formol solution (70 per cent " formalin " adjusted to pH 8·5 with 5 per cent sodium hydroxide) was added to each sample and the latter then back-titrated to pH 8·5 with 0·05 N sodium hydroxide solution. The back-titration varies according to the extent to which the ionising groups react to produce H^+ and, consequently, is a measure of proteolysis. The results, recorded in Table 4, indicate that the *manicotto* possesses significant proteolytic activity effective within the tryptic pH range.

Table 4—Proteolytic activity is expressed as mls. of 0·05 N NaOH corrected against controls. This applies to subsequent tables, also.

Species	Stages of development	No. of specimens per batch and number of batches	Incubation period (hrs.)	Fore-gut region	Proteolytic activity
Rana temporaria	53–55	18 (6)	72	*a*	0
				b	0·84
				c	0·01
Xenopus laevis	54–58	40 (5)	96	*a*	0·02
				b	1·72
				c	0·04
Bufo bufo	52–59	25 (8)	96	(*a–c*)	0·01

A further sample of the casein digest, with a few drops of toluene added to prevent putrefaction, was incubated for eight days and then tested for digestion products. Positive results were obtained for free and combined tryptophan (bromine test and the Hopkins-Cole-Adam-Kiewicz reaction) and for tyrosine. This suggests that the *manicotto* secretion is not purely tryptic but contains some peptidases, possibly carboxypeptidases. It is also of interest that, for the period during which food is normally retained in the larval gut ($\div 4$ hours) the proteolytic activity of the *manicotto* is of the same order, per unit weight, as that of the premetamorphic pancreas. This was shown by two methods. Firstly, by comparing the proteolytic activities of ethanolic extracts of the two tissues by means of formol titrations (Table 5). Secondly, by diluting 1 ml. samples of both extracts with distilled water to give 1/2, 1/4, 1/8 etc. of the original strengths—the final volume in each case being maintained at 1 ml. The diluted extracts were separately incubated with casein solution (10 ml. per sample) at 38°C for eight days and then tested by aldehyde reaction for tryptophan. The greatest dilution at which the yellow mercury compound of tryptophan could be discerned was taken as an index of the proteolytic activity of the particular extract employed. Pancreas extract gave a value of

128 (dilution of 1/128) and that of the *manicotto* 64 (dilution of 1/64). The difference represents a dilution factor of one and, since it occurs high on the dilution scale, the proteolytic values of the two extracts may be regarded, for the purposes of the present investigation, as equivalent. For this last experiment, *Xenopus laevis* tadpoles, only, were employed.

Table 5

Species	Stages of development	No. of specimens per batch	No. of batches	Mean value of *M*/P	Hours of incubation	Proteolytic Activity		
						Manicotto 20°C	*Manicotto* 38°C	Pancreas 38°C
Rana ridibunda	53–57	22	5	0·91	0·25		0·80	0·80
					1·5		0·92	0·96
					3·0		1·20	1·38
					6·0		1·20	1·44
					24·0		1·22	1·48
					18·0		1·20	1·48
Xenopus laevis	52–57	30	8	0·82	3·0	0·79	1·28	1·46
					72·0	0·91	1·30	1·58
					96·0	1·44	1·60	1·96
					120·0	1·42	1·68	2·00

Abbreviations : *M*/P, ratio of the weights of *manicotto* and pancreatic tissues.

The possibility of a one-way or reciprocal booster action occurring between the pancreatic and *manicotto* secretions was considered and investigated by incubating a mixture composed of equal volumes of the two extracts and estimating the resulting proteolytic activity by formol titrations. If booster action occurs, the titre values of the mixed extracts should be significantly higher than those of either extract alone. The results are recorded in Table 6, from which it is concluded that no boosting occurs but that the two extracts act in a simple, summative manner.

Table 6.

Species	Stages of development	No. of specimens per batch	No. of batches and hours of incubation	Composition of digest	Activity
Rana ridibunda	54–58	20	4 (12 hrs)	(P)	1·56
				($\frac{1}{2}$P+$\frac{1}{2}$*M*)	1·52
				(*M*)	1·44
Rana temporaria	56–59	25	5 (8 hrs)	(P)	0·67
				($\frac{1}{2}$P+$\frac{1}{2}$*M*)	0·70
				(*M*)	0·66
Xenopus laevis	50–60	15	10 (24 hrs)	(P)	1·52
				($\frac{1}{2}$P+$\frac{1}{2}$*M*)	1·50
				(*M*)	1·45

Abbreviations : P. pancreas extract ; *M*. *manicotto* extract.

Typically, the gut region occupied by the *manicotto* in the larva, becomes incorporated into the non-zymogenous, mucus-secreting duodenal end of the definitive stomach (*vide supra*). The histological reconstitution involved in this process proceeds antero-posteriorly so that, theoretically, it is possible for the anterior-most gastric glands to be formed before the most posterior of the *manicotto* elements have completely degenerated. Serial sections of some metamorphic stages suggest that this does occur. The pH optima of their respective enzymes, however, preclude the functional co-occurrence of the two glands. It is, consequently, of interest to know when the activity of the *manicotto* ceases and that of the gastric glands begins.

Manicotto activity was determined, as above, by formol titration. Peptic activity was calculated by the modification of the Volhard & Löhlein technique suggested by Barrington (1946). Twenty *Rana ridibunda* tadpoles were extracted for each stage and the proteolytic activities expressed as mls. of 0·05 N sodium hydroxide solution. The *manicotto* secretion ceased to be active immediately before the larval jaws are lost, when the gut epithelium is beginning its first slough. This is followed by a period (at 20°C) when the fore-gut produces no proteolytic secretion and the animal does not feed. At about the third day of the fasting period, histological differentiation of the

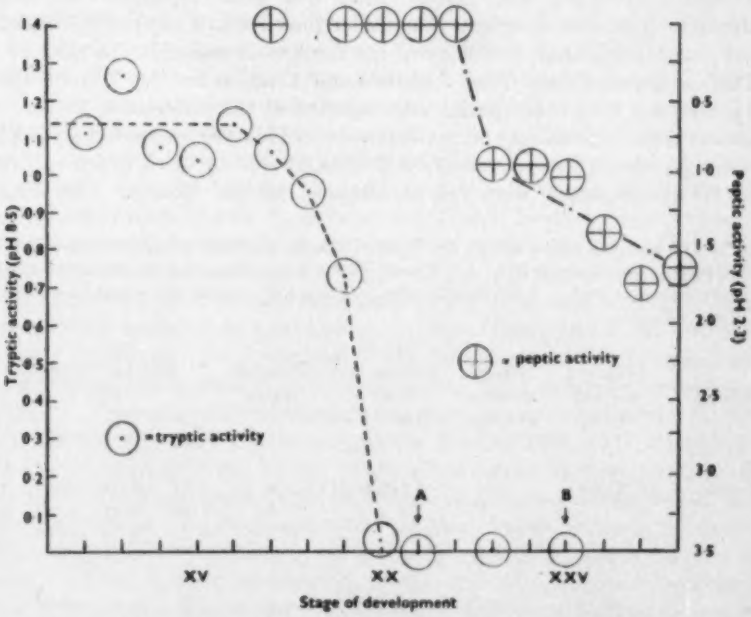

Fig. 13—Fore-gut proteolytic activity in larval and early post-metamorphic stages of *Rana ridibunda*. Activity values are expressed as mls. of 0·05N NaOH. Development is staged according to the Taylor & Kollros scale (1946). A and B, respectively, indicate the stages at which the larval jaws are lost and the tail completely resorbed. (Key to lettering, p. 282).

gastric glands begins. The first positive peptic test, however, is not recorded for a further three days—two days before feeding recommences. Animals were kept until the tail vestiges were lost and then for a further four weeks. The results are graphed in Fig. 13.

B. *The effect of diet on the fore-gut*

Yung (1904, 1905) and Babak (1905, 1911) concluded that differences in length and form of tadpole intestines is a function of the type of food taken during each ontogeny and that the extreme length of the intestine in larval Anura results from, and is an adaptation to, a plant-eating habit. Babak (1906) claimed to have induced a 58·15 per cent difference in the gut lengths of two tadpole batches, belonging to the same species, by rearing one on an exclusively vegetarian diet and the other on meat. Kunz (1924) supported the Yung-Babak hypothesis. His own researches (later supported by Dorris, 1935) demonstrated that a true pepsin-secreting stomach is present in larval urodeles and he suggested that this is correlated with the carnivorous habits of these forms. The same view had earlier been propounded by Ratner (1891) and was later endorsed by Bowers (1909); it was accepted, also, by Noble (1931). More recently Løvtrup (1955) has suggested that enzymes may "be synthesized as a response to the uptake of food". So far as the author is aware the experiments of Yung and Babak have not been repeated. It seemed particularly desirable, therefore, to consider the effects (if any) of different diets on the morphology and physiology of the fore-gut in salientian larvae.

The species used were *Rana ridibunda* and *Xenopus laevis*. Two batches of thirty tadpoles from each species were selected at the pre-feeding stage. One batch was reared exclusively on nettle powder (which was renewed daily) and the other on powdered liver (Deansely & Parkes, 1945). A third batch of twenty *Rana ridibunda* larvae were fed on chopped sheeps' hearts. The tadpoles

Table 7—The activity values for X, for *Rana ridibunda*, represent titration values in mls. of 0·05 N NaOH, whilst those of P, L, and M were obtained by subtracting the titre values of these categories from that of X. Similarly, for *Xenopus laevis*, P records the actual titre value and L the titre difference.

Species	Stages of development	No. of specimens per batch	No. of batches and hours of incubation	Fore-gut region	Diet and proteolytic activity			
					X	P	L	H
Rana ridibunda	47–54	20	5 (48 hrs)	*a*	0·01	–0·01	–0·01	0
				b	1·31	0·01	–0·02	–0·03
				c	0	0	–0·01	0·02
Xenopus laevis	49–58	35	4 (45 hrs)	*a*		0	–0·02	
				b		1·47	–0·03	
				c		0	0·01	

Abbreviations: X. extracts prepared from tadpoles collected in the wild; P. L. H., extracts prepared from tadpoles reared, respectively, on nettle powder, liver powder and chopped hearts.

were allowed to develop up to the late limb bud stage (stage 53, Nieuwkoop & Faber 1956) when they were killed and the general form, length and degree of coiling etc. of the guts noted. Representative specimens were fixed for histological examination and others were used to prepare extracts for peptic and tryptic analyses.

No significant intraspecific differences were observed in either gut length or spiralization pattern between the different larval batches. In the case of *Rana ridibunda* these points were further checked against tadpoles of a comparable stage of development collected in the wild. Histological examination also failed to demonstrate any differences between the various experimental groups. Finally, analyses of gut extracts showed that physiological differences, also, were absent : no pepsin was recorded and, for each species, titration values for tryptic estimations were of the same order for the different batches (Table 7).

DISCUSSION

The above results show that, both developmentally and functionally, the *manicotto* is a part of the larval pancreatic complex.* They show, also, that a true stomach occurs in none of the tadpoles examined, and the range of genera and species investigated (Table 1) suggests that this may be a characteristic feature of anuran larvae in general.

Despite its absence from all the bufonid and pelobatid species (Table 1) the distribution of the gland is clearly not a correlate of phylogenetic relationships since, in other families (Discoglossidae, Leptodactylidae, Ranidae and Rhacophonidae) its presence varies even intragenerically. Its presence is also unrelated to any particular type of feeding habit or feeding mechanism for although it occurs in some filter feeders (Pipidae, Microbylidae) it is absent from others (*Megophrys major*, *M. pelodytoides*) ; it is also absent from the larvae of *Rana limnocharis*, *R. luctuosa* etc. (Table 1) whose diet and feeding apparatus differ in no way from those of the more typical ranid forms in which a *manicotto* invariably occurs (Table 1). A solution to the distributional anomaly posed by the *manicotto* is suggested by a comparative consideration of the pancreatic complex in craniates generally. The gland occurs in one of two main conditions : (*a*) embedded in the intestinal wall or (*b*) as a discrete extra-intestinal organ which communicates with the gut lumen by means of a duct only. Type (*a*), which is unquestionably the more primitive (Brachet, 1897 ; Barrington, 1957), occurs in Agnatha (Maskell 1930, 1932 ; Barrington, 1936) and in Dipnoi (Kerr, 1919). Type (*b*) is found in most gnathostomes (including the non-manicottid anuran larvae) and, during development, arises as proliferations of the intestinal wall. It thus passes through a condition which, topographically at least, resembles the definitive gland of (*a*). The anlagen so formed then separate from the gut to produce condition (*b*). Immediately prior to the complete separation of the primordia from the gut wall the gland passes through a stage when it consists of three well defined regions : (*i*) an extra-intestinal mass representing the greater part of the gland ; (*ii*)

* Throughout this discussion, references to the pancreas should be interpreted as referring only to the exocrine portion of the typical gland.

residual anlagen elements still situated in the intestinal wall and (*iii*) a narrow bridge connecting the two regions. Normally this stage is brief. If, however, by a change in relative growth rates of the different components, this condition were protracted so that (*ii*) do not leave the gut wall but become functional *in situ*, a condition would result which would be identical with the pancreas/*manicotto* pattern of the tadpoles of *Xenopus laevis*, *Heleophryne rosei* etc. (Fig. 6 ; Pl. 4, fig. 15), and severance of the pancreas/*manicotto* bridge would result in the more typical *manicotto* condition of the ranids. It is suggested, therefore, that the presence or absence of a *manicotto* in larval anurans is explicable in terms of growth rate variations within a common developmental pattern. That such developmental deviations can occur in closely related forms is evidenced by the recent work of Steinbock & Anserhofer (1950) who showed that in one and the same turbellarian species, *Prorhynchus stagnatalis*, variations can occur in both cleavage pattern and sequence of organ formation without any distinguishable differences being observable in the adult. The production, by *Nereis dumerili*, of planktonic and nereidogenic larvae, both of which give rise to absolutely identical adult stages, is also well known. The intensity of such deviational changes which will be tolerated, will be a direct function of the degree of metamorphosis that the species undergoes. Where the latter is high, larval organs may be formed having neither functional nor structural continuity with those of the adult, since the latter are effectively constituted *de novo* at metamorphosis. In such larvae deviational tolerance, also, will be high. This is essentially the situation in the Anura where " adult and larva live in two different worlds " (Dunn, 1924) and selection at the pre-metamorphic level can, to a large extent, proceed independently of that of the adult. Urodeles, on the other hand, have virtually no metamorphosis and the larval organ is an integral stage in the development of that of the adult. This necessitates both morphological and functional continuity and, consequently, deviational tolerance will be low. This may explain why peptic activity is detectable in caudates even in pre-feeding stages (Løvtrup, 1955) for in this group the feeding and digestive mechanisms of the adult must determine the nature of those of the larva. Why the deviational tolerance which is available to anuran larvae should result in the production of a *manicotto* in some species but not in others is not clear, for, *a priori*, a pancreatic complex where all the tissue is compacted into a discrete gland, need be neither more nor less adapted to a particular feeding mechanism or a particular type of diet than one where some of the elements are retained and proliferate within the limits of the gut wall. And this is supported by the evidence of gut analyses in the different species (Table 1). The only interpretation possible, at present, is that the nature of the fore-gut is linked with another larval character whose selective value varies according to the species concerned.

The absence of a true stomach from the tadpoles of all species hitherto examined, suggests that the condition is primitive, for it is unlikely that a secondary trend would have become so uniformly established in an Order whose larvae display such diversity of form, habits and habitats. Kunz (1924) suggested that the absence of a peptic stomach is directly correlated with a vegetarian diet. This argument is certainly inapplicable to anuran larvae

many of whom e.g. *Rana rugulosa* (Smith, 1917), *R. cyanophlyctis*, *R. tigrina* (McCann, 1932,) *Ooeidozyga laevis*, *O. lima* (Smith, 1916 and present investigation), are completely carnivorous. Barrington (1946), chiefly on theoretical grounds, suggested two possible reasons for the absence of gastric digestion in anuran larvae : (*i*) the possible role of bacteria in the digestive mechanisms of tadpoles and, consequently, the need to provide a medium free of gastric acidity and (*ii*) the fundamentally microphagous feeding habits of the group. Although definite information is still lacking on this point, the extremely rapid rate (four to five hours) at which food passes through the gut in anuran larvae, seems, *a priori*, to argue against the first proposition. With regard to the second suggestion, cogent reasons, unquestionably, have been mustered for correlating the evolution of a true (peptic) stomach in vertebrates with the substitution of a macrophagous feeding habit for the more primitive microphagous one (Barrington, 1942). And, primitively, anuran larvae must be regarded as filter feeders. Some of their most characteristic features can be interpreted only in this way : many (including members of the more primitive families) are edentulous and where teeth and beaks do occur, they have clearly evolved (and in many cases still function) as extra-intestinal triturating mills and, consequently, as adaptations to microphagy. The oesophageal valves (" Filterklappe " of Kratchowill, 1933) and mucous feeding cords also point to a primitive filter-feeding mechanism ; the complex branchial apparatus (which in many species is only incidentally respiratory and often completely devoid of gills) suggests the same conclusion. For the majority of anuran larvae, therefore, it would seem valid to argue that the absence of a true stomach is, as Barrington suggested, a correlate of their microphagous feeding habits and, as such, no more problematic than the agastric condition of *Glossobalanus* (Barrington, 1940), *Tethym* (Berrill, 1929, *Amphioxus* (Barrington, 1937) or the ammocoete larva (Barrington, 1936). This explanation cannot, however, account for those species (*Rana rugulosa*, *R. tigrina*, *R. cyanophlyctis* etc.) which have adopted extreme macrophagy but not evolved a stomach and, particularly, is it inapplicable to such macrophagous forms as *Ceratophrys laevis* and *Ooeidozyga laevis* which have developed *non-gastric*, fore-gut storage chambers. The answer to the problem may be found in a suggestion made by Szarski (1956) and Szarski *et al* (1956). These authors explain the absence of a stomach in cyprinids by pointing out that these fish consume food rich in buffering salts, particularly carbonates. They emphasize that the elimination of a stomach in such forms results in a great economy of chlorine and phosphorus ions. A similar explanation may be proposed to account for the absence of peptic digestion in anuran tadpoles. The latter often inhabit pools whose waters and substrate are particularly rich in carbonates and, being generally indiscriminate feeders, they must pass large quantities of such salts passively through the gut. If their digestive system involved a peptic digestive phase the amount of hydrochloric acid which would have to be produced to overcome this basicity factor and maintain the pH at two to four, would be extremely high. The position would be further aggravated by the fact that the chloride ion is typically the rarest anion occurring in fresh water. It is suggested, therefore, that this problem of anion economy in anuran larvae outweighed any selection

pressure to evolve an acid stomach and the consequent establishment of a peptic digestion phase. That such pressures towards the establishment of fore-gut storage chambers have occurred, within the group, is indicated by the fore-gut modifications in *Ceratophrys laevis* etc. These larval chambers may, consequently, be interpreted as structures which parallel the stomach of the adult in storage function but differ from it in the pH range within which it operates. It is interesting to note that whilst, as in the case of vertebrates generally, the evolution of the fore-gut chamber in the anuran larva, also, is typically correlated with a macrophagous feeding habit it may in some instances, as in *Philautus gryllus*, be directly associated with a microphagous mechanism.

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EXPLANATION OF THE PLATES

Plate 1

Fig. 1.—*Rana ridibunda*. T. S. *manicotto* showing ciliated epithelium (Rugh stage 28).
„ 2.—*Rana ridibunda*. T. S. showing crypt system of definitive *manicotto* (Stage XIV, Taylor and Kollros).
„ 3.—*Rana ridibunda*. T. S. larva (Rugh stage 23) showing the origin of the *manicotto* cells.
„ 4.—*Rana ridibunda*. T. S. larva (Rugh stage 26) showing atrophying *manicotto*/pancreatic bridge.

Plate 2

Fig. 5.—*Calluela guttulata*. T. S. definitive *manicotto* showing reduction of epithelial area and complexity of crypt pattern. (Taylor & Kollros larval stage XV).
„ 6.—*Rana ridibunda*. T. S. degenerating *manicotto* showing syncytial epithelial and gland masses (Taylor and Kollros stage XXI).
„ 7.—*Rana ridibunda*. T. S. larva (Taylor and Kollros stage XXII) showing giant mitotic figures and pigment nests of degenerating *manicotto*.

Plate 3

Fig. 8.—*Rana ridibunda*. T. S. final stages in the degeneration of the *manicotto*. Note that no dedifferentiation is occurring. (Taylor and Kollros stage XXIII).
„ 9.—*Rana ridibunda*. (Stage XX, Taylor and Kollros). T. S. *manicotto* showing the round cells and one of the few patches of ciliated epithelium persisting at this stage.
„ 10.—*Rana ridibunda*. T. S. fore-gut showing the syncytial bands formed by the basal cells of the epithelium. (Stage XXIV Taylor and Kollros).
„ 11.—*Rana ridibunda*. T. S. stomach region showing the formation of the gastric gland by involution of the reconstituted epithelium.

Plate 4

Fig. 12—*Philautus gryllus*. Larval fore-gut (Stage IX, Taylor and Kollros).
„ 13.—*Philautus gryllus*. L. S. fore-gut diverticulum (Stage IX, Taylor and Kollros).
„ 14.—*Alytes obstetricans*. T. S. *manicotto*. (Larva stage X, Taylor and Kollros).
„ 15.—*Xenopus laevis*. L. S. *manicotto*. Larva stage 54 (Nieuwkoop and Faber).

Plate 5

Fig. 16.—*Philautus gryllus*. T. S. level 4, text-fig. 11.
„ 17.—*Philautus gryllus*. T. S. level 5, text-fig. 11.
„ 18.—*Philautus gryllus*. T. S. level 1, text-fig. 11.

Plate 6

Fig. 19.—*Philautus gryllus*. T. S. level 3, text-fig. 11.
„ 20.—*Philautus gryllus*. T. S. level 2, text-fig. 11.
„ 21.—*Rana ridibunda*. T. S. *manicotto* showing gland spheres rupturing the epithelium (Rugh stage 30).

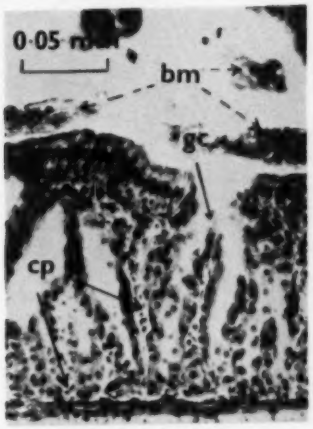
Figs. 16–20 are of stage V larva (Taylor and Kollros).

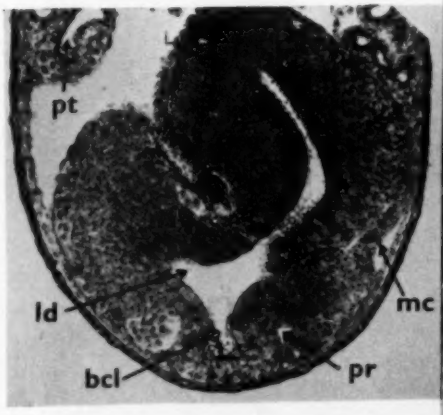
KEY TO THE LETTERING OF THE FIGURES

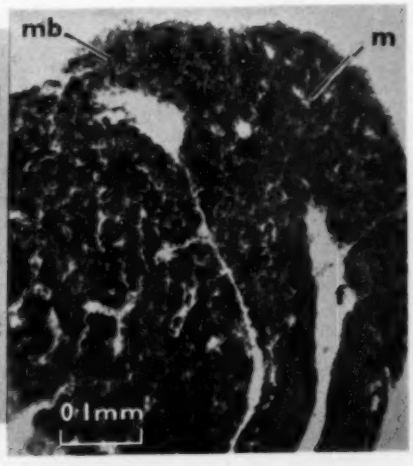
ag	Anti-*manicotto* grooves.	**cg**	Collecting grooves.
b	*Manicotto*-pancreatic bridge.	**cl**	Ciliated border of crypt opening.
bd	Bile duct diverticulum.	**cm**	Constrictor muscle.
bg	Basal (serosal) gland elements.	**cp**	Capillaries.
bm	*Manicotto* tissue budded into gut lumen.	**cv**	Cavity of diverticulum.
bt	Bifid muscular tongue.	**d**	Distal opening of hepato-pancreatic duct.
C	*Manicotto* crypt.	**dc**	Discharge point for degenerating gland cells.
cc	Lateral ciliated canals.		

KEY TO THE LETTERING OF THE FIGURES—*continued*

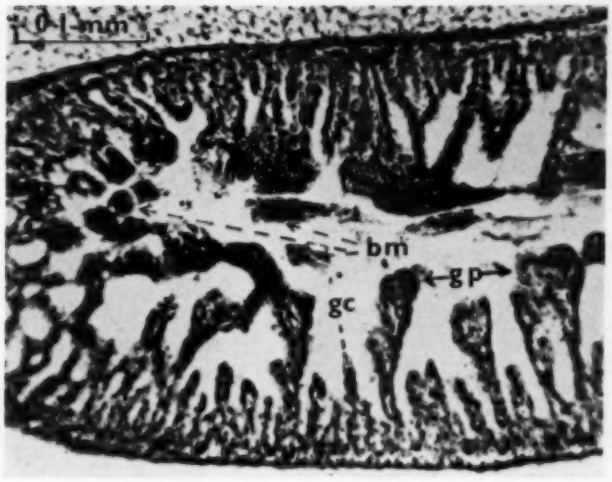
dm	Core musculature of diverticulum.
dn	Nuclei being discharged into gut lumen.
dp	Dorsal pancreas.
dv.	Diverticulum.
e	Point of entry of hepato-pancreatic duct into mid-gut diverticulum.
eg	Gland spheres rupturing *manicotto* epithelium.
es	Epithelial syncytium.
f	Fore-gut.
fc	Fore-gut cavity.
fg	Ciliated collecting grooves.
fi	Fore-gut involution.
fp	Fore-gut plug.
ft	Fore-gut tongue.
g	Gall bladder.
gc	Gland cord.
gp	Epithelial gap.
gr	Glandular rupture-point in the epithelium.
h	Hemisphincter.
hp	Hepato-pancreatic duct.
i	Intestine.
io	Lateral opening of fore-gut tongue.
J	Post-*manicotto* fore-gut.
k	Muscular capsule of the *manicotto*.
kc	Mitosis.
L	Gut lumen.
l	Gland lumen.
lb	Left lung bud.
ld	Liver diverticulum.
lv	Liver.
m	*Manicotto*.
mb	*Manicotto*-pancreatic bridge.
mc	*Manicotto* cells.
mch	*Manicotto* chamber.
md	Mid-gut diverticulum.
mf	*Manicotto* flap.
mg	*Manicotto* flange.
mj	*Manicotto*-pancreatic junction.
mt	Muscle of fore-gut tongue.
o	Oesophagus.
oc	Opening into *manicotto* crypt.
og	Outer generative layer.
op	Oesophageal plug.
P	Anterior limit of *manicotto*.
p	Pancreas.
p(i) ; p(ii) ;	pancreatic lobes.
pg	Primary groove.
ph	Pharynx.
pl	Distal limit of hemisphincter.
pn	Pigment nest.
pr	Pancreatic anlage.
pt	Pronephric tubules.
Q	Distal limb of fore-gut.
R	Anterior limit of fore-gut tongue.
r	Recurrent hepato-pancreatic ductules.
rr	Reservoir.
rc	Round cells.
re	Reconstituted epithelium forming gastric glands.
rm	Circular muscle.
S	Posterior limit of *manicotto*.
s	Secretory granules.
sb	Subintestinal blood vessel.
sm	Serosa and attenuated muscle layer.
sn	Syncytial nest of degenerating gland.
sp	Sphincter.
sq	Secondary groove.
t	Transverse septum.
tm	Transverse muscles.
U	Proximal limb of fore-gut.
vp	ventral pancreas.
W	Gap between the basal cell bands through which degenerating gland tissue is discharged.
Y	Degenerating epithelial and *manicotto* tissue being sloughed into the gut lumen.
Z	Syncytial band.

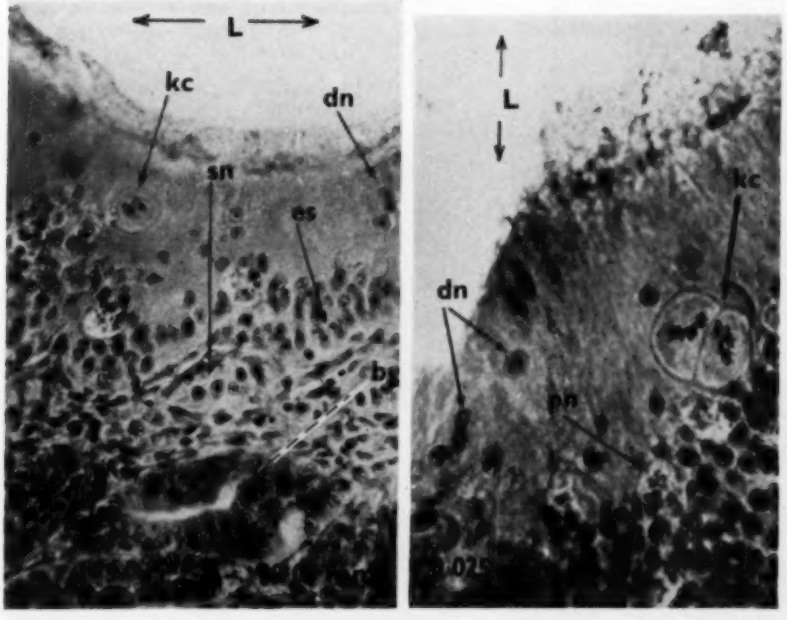




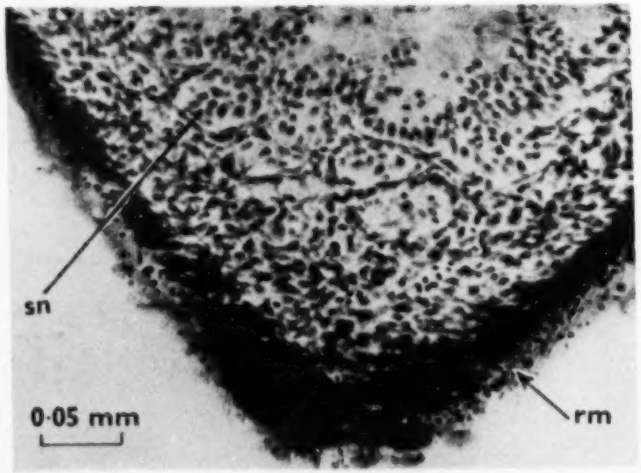


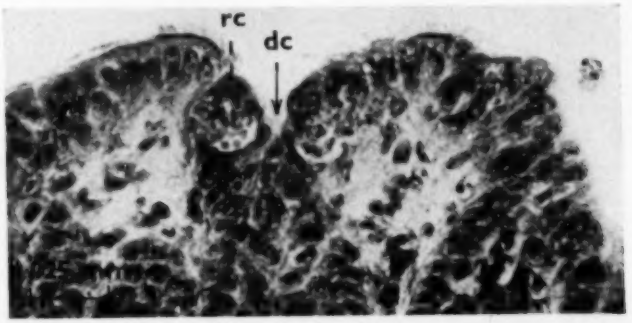
The form and function of the fore-gut in anuran larvæ.

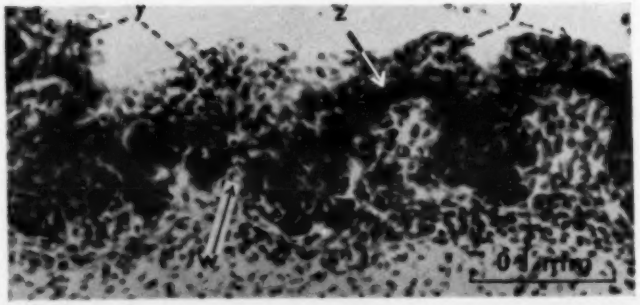


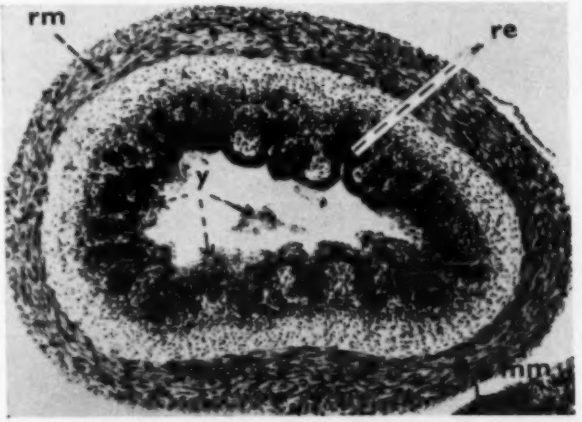


The form and function of the fore-gut in anuran larvæ.

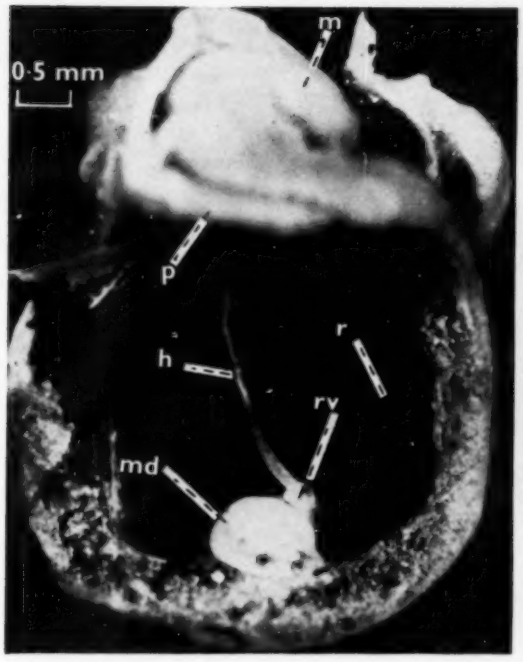




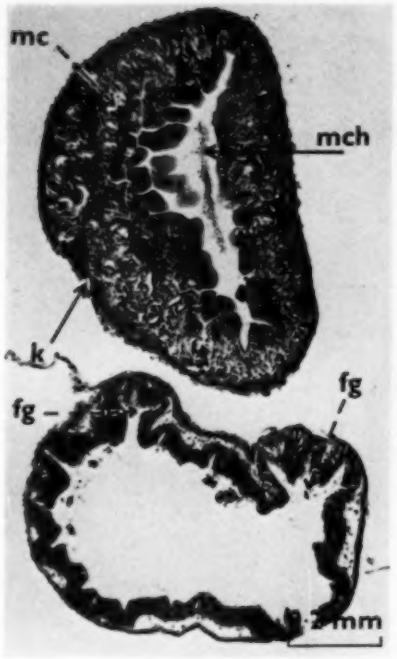


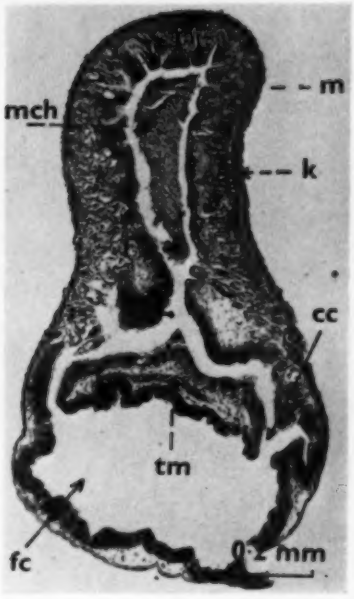


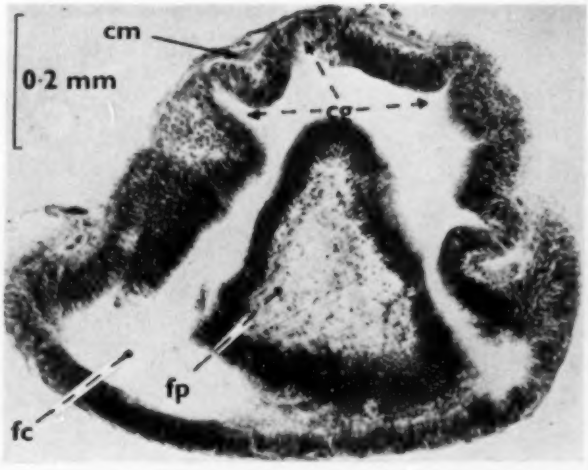
The form and function of the fore-gut in anuran larvæ.



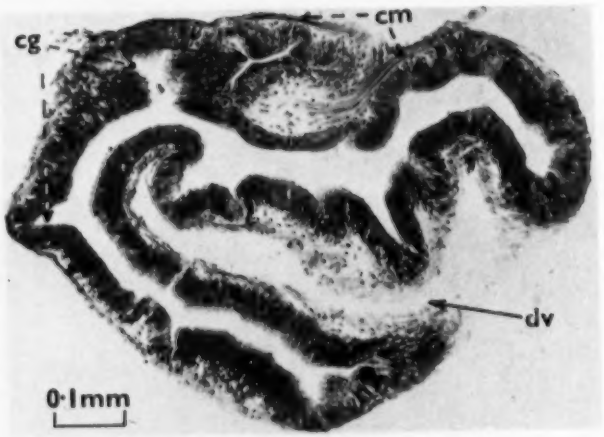
The form and function of the fore-gut in anuran larvæ.

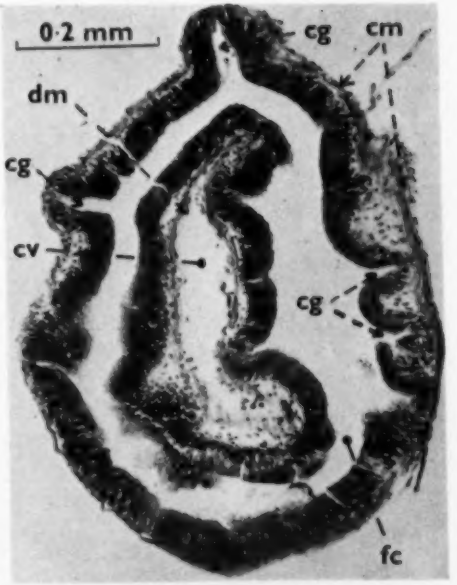






The form and function of the fore-gut in anuran larvæ.





The form and function of the fore-gut in anuran larvæ.

THE EVOLUTION OF THE MAMMALIAN FEMUR

BY

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[Accepted 14th February, 1961]

(With 9 figures in the text)

Comparison of the femora of advanced cynodonts, tritylodonts and monotremes shows that the antero-ventral trochanter of the cynodonts is the mammalian trochanter minor and not the reptilian trochanter internus as has been held. Consideration of the form of the pelvis supports this conclusion.

The femora and pelves of anomodonts indicate that an essentially mammalian musculature was developed and that, though no trochanter minor is known in the group, an iliacus muscle must be presumed to have been developed.

Among Theriodontia, the gorgonopsids are considered to have retained a largely reptilian thigh musculature, but the therocephalia and scaloposaurid bauriamorphs appear to have achieved a condition intermediate between those of reptiles and mammals.

It is claimed that the femora of primitive Theria are very similar to those of advanced cynodonts and could have been derived directly from one of this type. The femora of tritylodonts and monotremes, and two of the three supposed mammalian femora from the Jurassic, are considered to be specialized.

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INTRODUCTION

Current views of the evolution of the mammalian femur and its various trochanters are based largely on the work of Gregory & Camp (1918) and of Romer (1922, 1924). By comparing the leg musculature of living Amphibia, reptiles and mammals, and examining the femora of various extinct reptiles, including mammal-like reptiles, they were able to demonstrate that the trochanter major arose within the therapsid forerunners of mammals and that the third trochanter is probably peculiar to mammals. Also Romer pointed out that although the reptilian trochanter internus occupies the same position on the bone as the mammalian trochanter minor, the two could not be homologous since the reptilian trochanter marks the insertion of part of the pubo-ischio-femoralis *externus* muscle, while the mammalian trochanter is for the insertion of the iliacus and psoas muscles which are derivatives of the reptilian pubo-ischio-femoralis *internus* muscle. In the course of his work

Romer identified an antero-ventral trochanter of cynodont* reptiles as the reptilian internal trochanter and this interpretation has been accepted generally. But the subsequent discovery of many more specimens, some of which are very well preserved, and in particular the discovery of the femora of two tritylodonts, makes this determination very difficult to accept.

Romer's (1924) account of the cynodont femur was based on the bone from the classical skeleton of *Cynognathus* which was described by Seeley (1895), and on a femur of *Diademodon*, but the former at least is very poorly preserved. The femora of the cynodonts *Cricodon metabolus* Crompton and *Scalenodon*

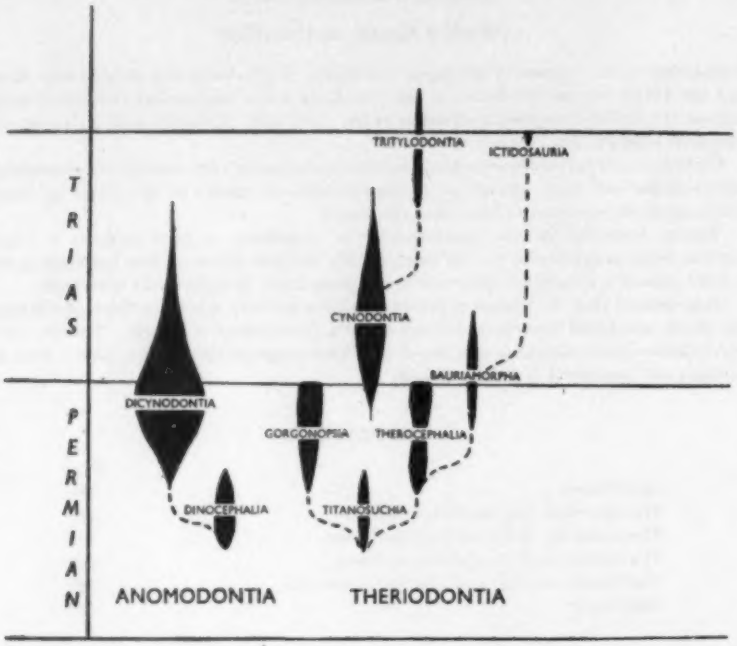

Fig. 1—Diagrammatic representation of the evolution of the Therapsida. The suborder Anomodontia consisted of herbivores; the suborder Theriodontia, which was ancestral to mammals, largely of carnivores.

angustifrons (Parrington), together with others from the Lower Trias of South Africa and the Middle Trias of Tanganyika, have characters which give the bone a much more mammalian appearance than did the material available to Romer. Contrary to his general account of the therapsid femur (Romer, 1956) the cynodont femur is a moderately slender bone and (unlike that of the

* It is convenient today to divide the synapsid, or mammal-like, reptiles into two orders, the primitive Pelycosauria and the more advanced Therapsida (Watson & Romer, 1956). The latter order comprises the herbivorous Anomodontia and the typically carnivorous Theriodontia which gave rise to the mammals. The various infraorders of this latter group and their probable relationships, are indicated in Fig. 1.

contemporaneous dicynodonts) is not flattened ; the head, which is set off at a variable angle to the shaft, becomes distinctly hemispherical ; the antero-ventral trochanter is usually strongly developed ; and there is often a distinct inter-trochanteric fossa.

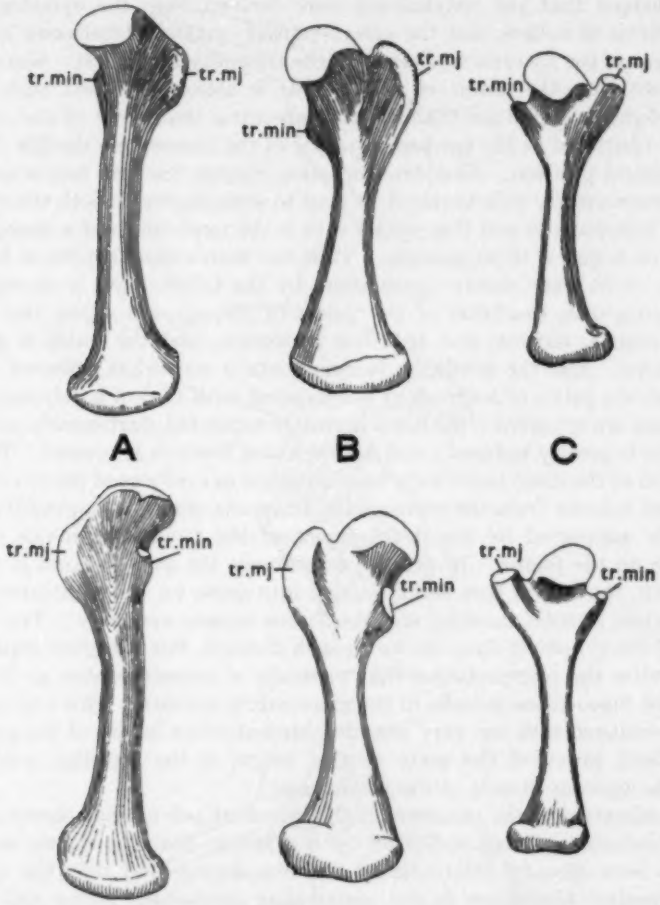

Fig. 2—Dorsal (upper figures) and ventral aspects of the left femora of A the cynodont *Cricodon* (M. Trias) ×½ ; B the tritylodont *Bienotherium* (U. Trias) ×⅔ ; C the tritylodont *Oligokyphus* (L. Jurassic) ×1. tr.mj., trochanter major ; tr. min., trochanter minor. B after Young, C after Kühne.

THE CYNODONT AND TRITYLODONT FEMORA

If the femur of the Middle Triassic cynodont *Cricodon* is compared with that of the tritylodont *Oligokyphus* from the Lower Jurassic (Kühne, 1956) certain differences are at once apparent (Fig. 2). In the tritylodont the trochanter

major has become separated from the head by a distinct neck, the antero-ventral trochanter of the cynodont is replaced by an anterior trochanter, and the distal condylar surface is ventral rather than terminal. But the femur of the tritylodont *Bienotherium* from the Upper Trias (Young, 1947) appears to be intermediate in all respects between the two and, since it is commonly acknowledged that the tritylodonts were derived from the cynodonts, it is very difficult to believe that the antero-ventral cynodont trochanter is not the homologue of the anterior trochanter of the tritylodonts (Fig. 2). Since Kühne has shown that the femur of *Oligokyphus* is almost identical with that of *Ornithorhynchus* it follows that the antero-ventral trochanter of the cynodont must be identified as the trochanter minor of the monotreme though it is in a more ventral position. This determination implies that the iliacus and psoas mucles were already differentiated, at least to some degree, in both the cynodont and the tritylodonts and this carries with it the implication of a change in the regions of origin of these muscles. That the mammalian condition had been reached, or at least closely approached, by the tritylodonts is shown by the very mammalian condition of the pelvis of *Oligokyphus* where the ilium is pre-acetabular, narrow, and trihedral in section, and the pubis is of minor proportions. But the condition in cynodonts is somewhat different (Fig. 3).

When the pelvis of a cynodont is compared with that of a pelycosaur three differences are apparent ; the ilium is greatly expanded, particularly anteriorly; the pubis is greatly reduced ; and an obturator fenestra is present. The great expansion of the ilium has always been accepted as evidence of the development of gluteal muscles from the reptilian ilio-femoralis muscle, a supposition which is clearly supported by the development of the trochanter major for their insertion on the femur. In primitive mammals the slender ilium is trihedral in section, the lateral face being divided into areas for the attachment of the deep gluteal muscles dorsally and the iliacus muscle ventrally. The anterior blade of the cynodont ilium shows no such division, but the great depth of the bone invites the interpretation that ventrally it accommodated an iliacus, or combined iliaco-psoas muscle, in the mammalian manner. This interpretation is in accordance with the very considerable reduction in size of the pubis, the bone which provided the main area of origin of the reptilian pubo-ischio-femoralis internus muscle in the pelycosaurs.

Consideration of the structure of the cynodont pelvis leads therefore to the same conclusion as that indicated by the femur, that the iliacus and psoas muscles were already differentiated to some degree, and that the cynodont antero-ventral trochanter is the mammalian trochanter minor and not the reptilian trochanter internus. The transition from the reptilian to the mammalian condition must, therefore, be sought at an earlier stage of therapsid evolution.

THE EVOLUTION OF THE ANOMODONT FEMUR

Romer has shown that the thigh musculature of the Pelycosauria must have been primitive. In these forms the ilium was not expanded and the pubis was large, approaching the size of the ischium. The femur, which had the head situated more or less terminally, had a Y shaped system of ridges on

the ventral surface with what appears to be reasonably interpreted as a reptilian trochanter internus on the anterior fork of the Y, and a rugosity on the postero-dorsal region which appears to be a special insertion for part of the pubo-ischio-femoralis internus muscle (Fig. 4). The evolution of the anomodont type of

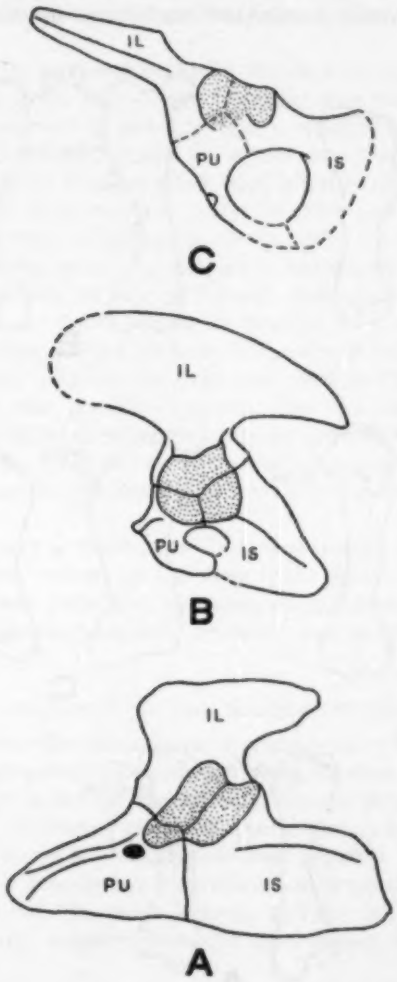

Fig. 3—Left aspect of the pelves of A the pelycosaur *Dimetrodon* (L. Permian); B the cynodont *Galesaurus* (L. Trias); C the tritylodont *Oligokyphus* (L. Jurassic). Not to scale.
IL. ilium; IS. ischium; PU. pubis.

femur from this condition appears to have been relatively simple. The early dinocephalian *Deuterosaurus* had a femur essentially like that of a pelycosaur, with a recognisable Y shaped arrangement of ridges ventrally. There is what

appears to be the beginning of a trochanter major and a distinct trochanter internus is still present on the anterior fork of the Y, but the fourth trochanter, for caudo-femoralis muscle insertion, is not obvious nor is there, apparently, any evidence of a special insertion for part of the pubo-ischio-femoralis internus muscle on the postero-dorsal surface. In the later Dinocephalia from South Africa the femur is usually stouter and has become flattened ; the Y shaped

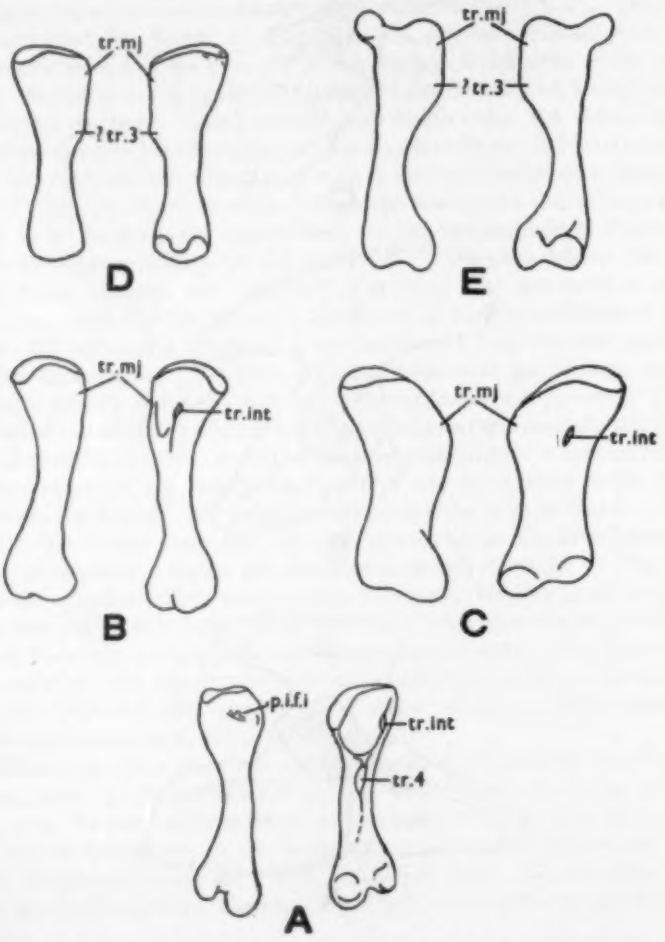

Fig. 4—Dorsal (left figure) and ventral aspects of the left femora of A the pelycosaur *Dimetrodon* (L. Permian) ; B the dinocephalian *Deuterosaurus* (M. Permian) ; C the dinocephalian *Struthiocephalus* (U. Permian) ; D the dicynodont *Dicynodon* (U. Permian) ; E the dicynodont *Shansiodon* (Trias). Not to scale.

tr.3., third trochanter ; tr.4., fourth trochanter ; tr.int., trochanter internus ; p.i.f.i., special insertion for part of the pubo-ischio-femoralis internus muscle. Other abbreviations as before. A after Romer and Price, B after Efremov. C after Boonstra, E after Yeh.

system of ventral ridges has gone, leaving a trochanter internus in only some forms ; and the trochanter major is clearly recognisable (Boonstra, 1955).

The dicynodont femur is still more flattened ; the trochanter major is well developed and is extended distally into what may have been an incipient third trochanter ; and there is no antero-ventral trochanter at all. A well developed head, set at a marked angle to the shaft, was achieved by the Triassic dicynodont *Shansiodon*.

The reduction of the reptilian trochanter internus among the dinocephalians, and its total absence in the dicynodonts, together with the development of the trochanter major, appears to indicate that a change from a reptilian to a mammalian condition started among the earlier members of the group. This inference is supported by changes which took place in the pelvis (Fig. 5). In the Dinocephalia the ilium shows a moderate anterior development and the pubis has undergone some reduction (Boonstra, 1955) ; in the dicynodonts the ilium is greatly expanded anteriorly and, where known, the pubis is of moderate size, being about one half, or less, of the size of the ischium. Moreover Cox (1959) has described the pelvis of the dicynodont *Kingoria* showing it to be exceedingly mammalian in form with the ilium almost entirely pre-acetabular and trihedral in form. The obvious inference, that in *Kingoria* an iliacus, or iliaco-psoas, muscle was present, suggests that the deep, but undivided, anterior part of the typical dicynodont ilium also served in part for the attachment of such a muscle. This conforms to the conclusion already drawn that the very similar ilium of cynodonts also provided for the attachment of an iliacus muscle.

It would appear very probable, therefore, that in spite of the absence of the mammalian trochanter minor on the femur, the thigh musculature of the dicynodont reptiles had undergone considerable modification and approached the condition found in mammals and, moreover, had reached this condition in the late Permian.

THE EVOLUTION OF THE THERIODONT FEMUR

The evolution of the theriodont femur appears to have been somewhat more complicated. Such primitive Titanosuchia as the Russian form *Titanophoneus* (Orlov, 1958) have more slender femora than typical pelycosaurs, and the Y shaped arrangement of ventral ridges is represented only by a single ridge, but postero-dorsally there is a roughened area possibly indicating a special insertion of part of the pubo-ischio-femoralis internus muscle as in pelycosaurs. The ilium is unexpanded, the pubis is large, and the tail long and powerful. This osteology strongly suggests that the musculature was still essentially reptilian.

Titanophoneus is extremely gorgonopsid-like and it is not surprising that the gorgonopsid femur is very like that of *Titanophoneus*. As shown by a series of seven femora of various sizes collected from Upper Permian deposits in the Ruhuhu Valley in Tanganyika, it is a slender, somewhat sigmoidally curved bone with the head set at an angle to the shaft, and there is only a moderately developed trochanter major (Fig. 6). Ventrally there is a well developed ridge extending from near the head to a point more than halfway down the

shaft of the bone. Several specimens show that this ridge has a moderate trochanter-like enlargement proximally, that it fades somewhat distally to this development but re-develops into a moderate trochanter below the level of the trochanter major, and then fades gently into the shaft of the bone more than halfway down its length.

It would appear that the proximal trochanter is best matched with the trochanter internus of the pelycosaurs and the more distal one with the fourth

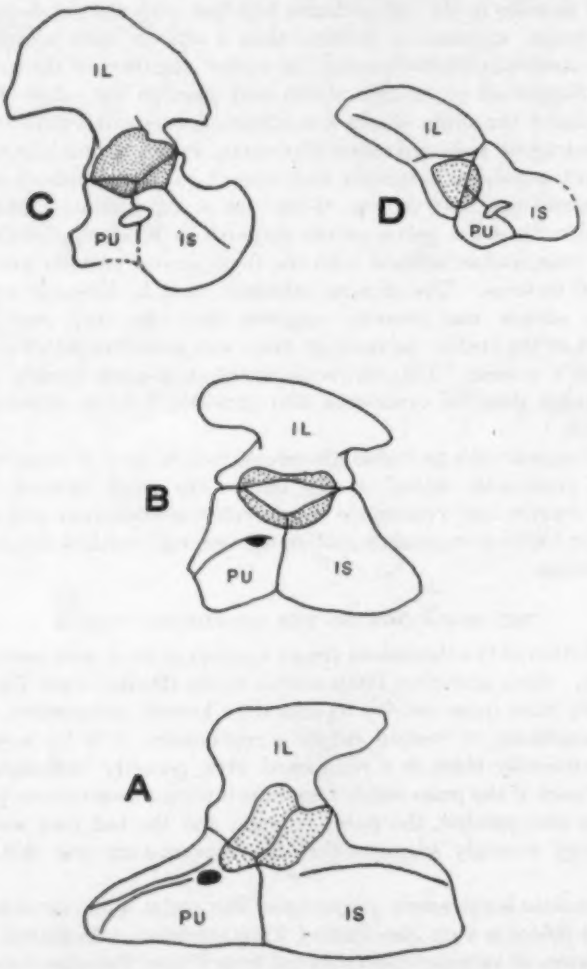

Fig. 5—Left aspect of the pelves of A the pelycosaur *Dimetrodon* (L. Permian); B the dinocephalian *Struthiocephalus* (U. Permian); C a Middle Triassic dicynodont; D the dicynodont *Kingoria* (U. Permian). Not to scale. A after Romer and Price, B. after Boonstra, D after Cox. Abbreviations as before.

trochanter, a conclusion which conforms to the opinion that the gorgonopsids, like the Titanosuchia, had substantial tails and must have retained something of the caudo-femoralis musculature of the pelycosaurs. The gorgonopsid pelvis has a slight expansion of the ilium, in conformity with the moderate trochanter major, and a substantial pubis in conformity with the retention of the trochanter internus. In general, therefore, the thigh musculature of the gorgonopsids must be held to have been largely reptilian.

Though many skulls of the contemporaneous Therocephalia, and their descendents the Bauriamorpha, have been described, no useful account of the femur has yet been given. Three specimens are available for this discussion.

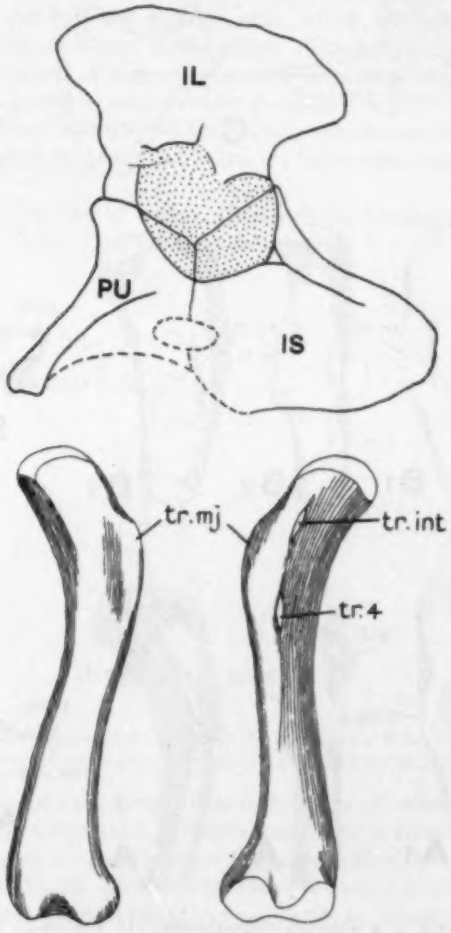

Fig. 6—Left side of pelvis and left femur in dorsal (left figure) and ventral aspect of unidentified gorgonopsids from the Upper Permian.

The first is the proximal half of a whaitsiid therocephalian from the Upper Permian deposits in Tanganyika ; the second is that of an as yet undetermined scaloposaurid bauriamorph from the L. Trias of South Africa ; the third is the proximal half of an isolated bone from the Upper Permian of Tanganyika. All three bones show essentially the same features (Fig. 7). The head is somewhat

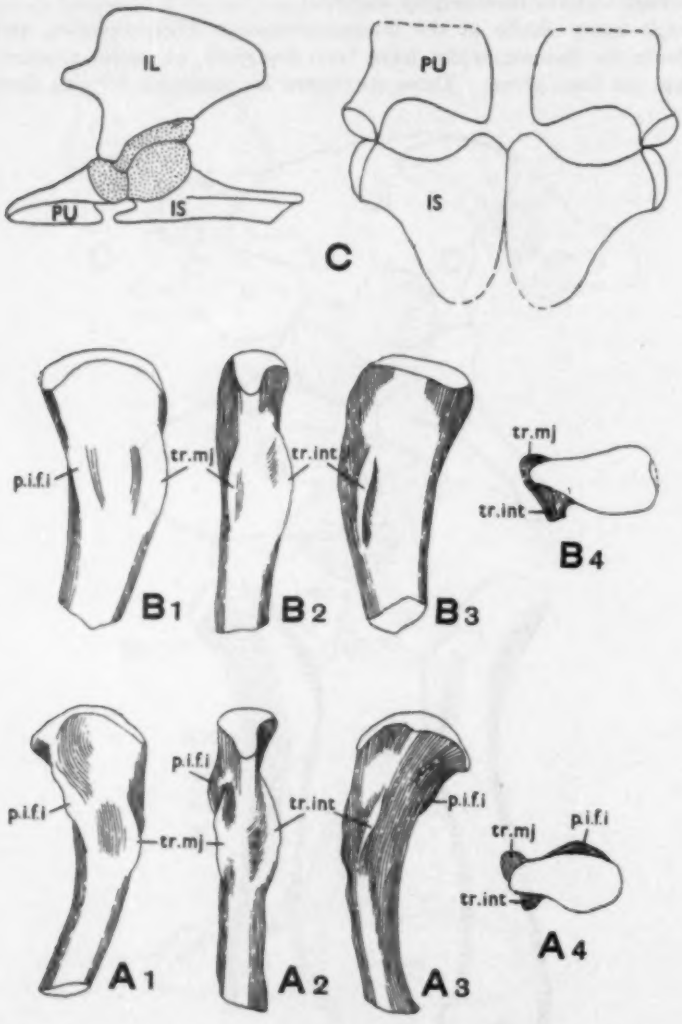

Fig. 7—A the left femur of a whaitsiid therocephalian (U. Permian) ; B the left femur of a scaloposaurid bauriamorph (L. Trias). C pelvis of the scaloposaurid *Ericiolacerta* (L. Trias). A1, B1 dorsal view ; A2, B2, posterior view ; A3, B3, ventral view ; A4, B4, proximal view. A × ½, B × 2½, C × 2 ; C after Watson.

expanded but there is no neck, the curve of the proximal part of the bone turning the head somewhat from the line of the shaft. A moderate trochanter major is present and ventrally there is a pronounced but short ridge which appears to represent the trochanter internus. Antero-dorsally there is a rounded ridge, more pronounced in the whaitsiid than the other two bones, which cannot be matched on the femora of either gorgonopsids or cynodonts. This ridge is in the same position as the supposed muscle scar for the special insertion of the pubo-ischio-femoralis internus muscle which Romer (1924) claimed on the femora of a dicynodont and a cynodont, but which are not recognisable on the well preserved material now available, and would appear to be interpretable in the same way. The femur would appear to be about intermediate between those of the gorgonopsids, which are largely reptilian, and the cynodonts, which are largely mammalian. This interpretation finds support in the form of the pelvis of the scaloposaur *Ericiolacerta* described by Watson (1931). Here the pubis is substantially developed but there is an obturator fenestra, and the ilium is expanded posteriorly but not anteriorly except for an antero-ventral projection which can be held to have accommodated an incipient iliacus muscle.

The therocephalian femur seems, therefore, to be about intermediate in structure between those of reptiles and mammals.

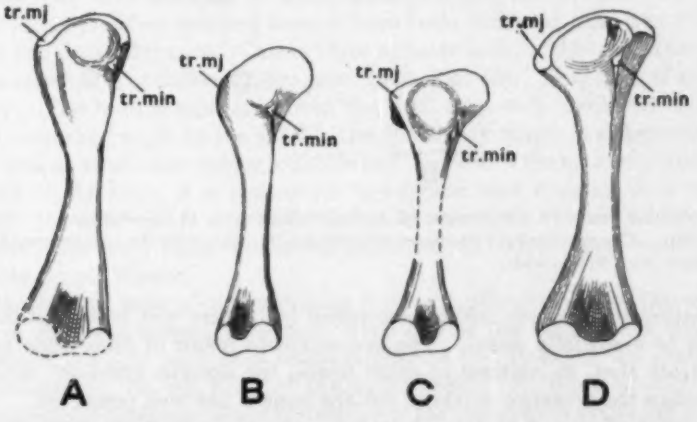

Fig. 8—Left femora of the cynodonts A *Galesaurus* (L. Trias) ×1 ; B *Scalenodon* (M. Trias) ×½ ; C ? *Aleodon* (M. Trias) ×½ ; D, unidentified cynodont (M. Trias) ×1½.

While the femora of cynodonts differ from those of other theriodonts, and appear to be clearly recognisable, nevertheless there is some variation within the group. Comparing the small femur of the primitive cynodont *Galesaurus*, from the L. Trias of South Africa, with those collected from the M. Trias of Tanganyika (Fig. 8) it appears that the head tends to become distinctly hemispherical and set at a greater angle to the shaft (*Cricodon*, *Scalenodon*). Moreover, while complete and undamaged bones are rare, it appears that the bone becomes somewhat twisted with the result that the dorsal (anterior) surface

of the distal end comes to lie at a more marked angle to the face formed between the head and the trochanter major ; this change is probably concerned with the developing angle of the head and indicates a more vertical position of the limb. The trochanter major is usually more clearly separated from both the head and the shaft in the later forms, and may be turned antero-dorsally (*Scalenodon*) or develop both anterior and posterior flanges (? *Aleodon*) and its position relative to the head is somewhat variable. The trochanter minor is usually stout but may be only moderate (*Cricodon*), and the median face of the proximal part of the shaft is usually flattened, but may be rounded. The distal

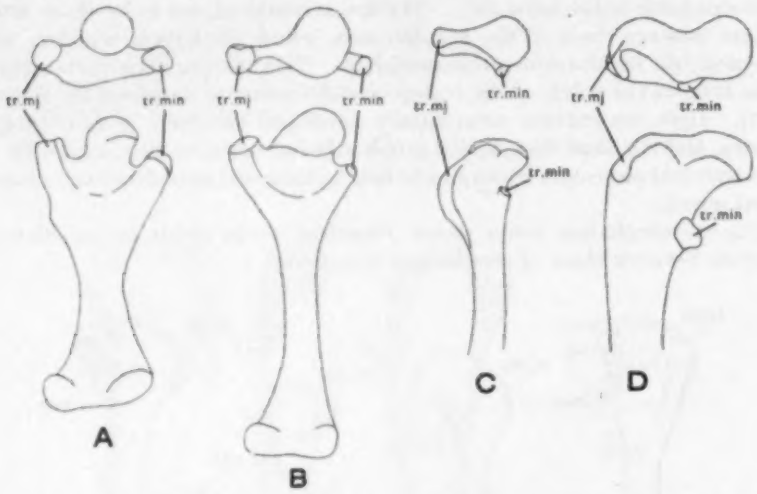

Fig. 9—Ventral views of the femora of A *Ornithorhynchus* ; B *Oligokyphus* ; C *Cricodon* ; D *Didelphis*. The cynodont (C) resembles the therian (D) rather than the monotreme (A) or the tritylodont (B). Not to scale.

articulating surfaces are seldom uncrushed but where well preserved they are seen to be essentially distal. The one complete femur of *Scalenodon* appears to indicate that, in contrast to other forms, the anterior (median) condyle is larger than the posterior condyle, but the bone is not well preserved.

In spite of this variation of form among the cynodonts (and a similar variation will no doubt eventually be shown in the other infraorders) the femora of the theriodonts fall into three categories : — the titanosuchids and gorgonopsids ; the therocephalia and primitive bauriamorphs ; and the cynodonts and tritylodonts. This grouping is in conformity with opinions of their relationships as indicated by other evidence. But, as interpreted in this account, the femur gives no clear evidence indicating the origin of the cynodonts from either the Gorgonopsia or the Therocephalia since no truly intermediate forms have yet been found. It might appear, however, that the mammalian condition of the cynodont bone is more closely approached by the therocephalian femur than by that of the gorgonopsids. It is unfortunate that nothing is

known of the femora of either advanced bauriamorphs or the ictidosaur *Diarthrognathus*.

THE FEMORA OF EARLY AND PRIMITIVE MAMMALS

If it is accepted that the antero-ventral trochanter of the cynodont femur is the mammalian trochanter minor and not the reptilian trochanter internus, and a comparison of the bone is made with the femur of a primitive therian such as *Didelphis*, they are found to be very similar. But both types contrast with those of the tritylodont *Oligokyphus* and the monotremes where the head is more terminal, the trochanter minor is anterior in position, and the distal condyles are situated ventrally rather than terminally (Fig. 9). It is clear from the angle of the head and the position of the distal condyles that the cynodont femur was held in a largely vertical position and not horizontally as in *Oligokyphus* and the monotremes. That the horizontal pose of the bone in these latter forms was secondary, and not the retention of a primitive condition, is clear from the form of the pelvis and the mammalian musculature of the monotremes. Direct derivation of the true therian femur from one resembling those of advanced cynodonts is therefore invited.

Only a single, isolated femur is known from the M. Jurassic. This was found at Stonesfield and, as Kühne (1956) has pointed out, it is of tritylodont form and may have belonged to the tritylodont *Stereognathus* which was found at this locality. Two isolated femora have been collected from the Purbeck Beds in the Upper Jurassic. One of these appears to be of the tritylodont type but the other is very different (Simpson, 1928. Fig. 49). This bone is exposed dorsally. The head is separated from the shaft by a well developed neck and held at a marked angle to the shaft ; the trochanter major is substantial and high ; and no trochanter minor is visible but, in view of the typically mammalian form of the bone, it is reasonable to suppose that it exists in a ventral position. In all respects this latter femur is essentially therian in form and its direct derivation from one of an advanced cynodont type would be a relatively simple matter.

In the present state of our knowledge it is very difficult to relate the various orders of Mesozoic mammals to each other and to the theriodonts, and any views which are held have been based largely on the nature of the dentitions and, particularly, on the form of the molar teeth. It would appear likely that a substantial increase in our knowledge of the postcranial skeletons of these animals would prove to be of considerable value in solving the taxonomic difficulties and thereby throw more light on the value of dentitions in such matters.

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GROWTH OF THE POSTLARVAL SHELL IN *LINGULA UNGUIS* (L.) (BRACHIOPODA)

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(With 1 plate and 5 figures in the text)

The growth of the postlarval shell of *Lingula unguis* on the north and east coasts of Singapore Island was studied. The youngest and the oldest postlarvae, observed over a period varying from one month to five years, grew in length and width all the year round. Length and width increments are inversely proportional to initial lengths and widths respectively, the linear negative correlation between increment and initial dimension being significant and high. Growth is more rapid on the north than the east coast. Postlarvae transplanted to the north coast made greater increments than those left behind to grow in their original burrows on the east coast. Possible factors responsible for this difference in the rate of growth between the two localities are suggested. The regression of width increment on initial width nearly parallels that of length increment on initial length after one as well as after three years' growth, indicating a similar rate of decrease of increment with increasing initial width or length. The transformation of the shape of the shell is reflected in the changing width/length ratio. The change in width/length ratio during the postembryonic development of the shell is a continuous process. This ratio drops from a range of 1·5–2·0 to around 0·8 during the larval stage and to around 0·4–0·5 by the time the postlarval shell length reaches 4 mm. During the rest of the postlarval life the ratio varies sufficiently to make this an unreliable specific character for *Lingula unguis*. The region of maximum width of the two shell valves in the same individual coincides but its relative position changes during the long postlarval stage. The shell length of the female at maturity, the maximum shell length in a sample and the longevity of *Lingula unguis*, are discussed.

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INTRODUCTION

During ontogeny the growth of the shell in *Lingula unguis* (L.) essentially consists of two discrete processes, namely the formation during the embryonic stage of the semi-circular embryonic shell and the subsequent deposition of the

postembryonic shell. The embryonic shell, first noted by Brooks (1878), was first recognized as such and named the protegulum in *Glottidia pyramidata* by Beecher (1891). The formation of the postembryonic shell is interrupted temporarily by a change from the planktonic larval life to the sedentary burrowing postlarval one at the time of settlement. This interruption produces a conspicuous disturbance ring marking off the approximately circular larval shell from the postlarval shell that forms after settlement. The larval shell was first recognized by Brooks (1878) on the outer surface of the young postlarval shell in *G. pyramidata*. Both protegula and larval shell valves persist for several years on the external surface at the umbo of the postlarval shell.

In *Lingula unguis* the postlarval shell consists of a pair of externally convex, opposed valves. New shell material is deposited in a thin layer on the inner surface of the existing shell, adding to its thickness and extending its margin at varying rates appropriate to both the site of deposition and the stage of ontogeny. The shell is thicker over the visceral cavity than the mantle cavity. The convexity of the shell valves increases during ontogeny. The varying curvature and thickness of the different parts of the shell rule out shell thickness as a character for measurement.

The use of the length and the width of the postlarval shell for the description of *Lingula* species dates from the time of Sowerby (1812) and the use of the proportion of the greatest width to length from that of Koninck (1842). Johnston & Hirschfeld (1920) regarded the length/width ratio in a species to be a fairly constant character which could be used for the separation of recent species that closely resemble one another. Wehrli (1931) and Deleers & Pastiels (1952), who noted the decreasing width/length ratios with increasing length of the shell in small specimens of the fossil, *Lingula mytiloides*, evidently encountered a growth series of young postlarvae.

In this paper the linear growth of the shell and the relation between width and length during ontogeny have been studied.

MATERIAL AND METHODS

Several easily identified plots about three by seven metres in size at the level of low-water neap tide in the *Lingula unguis* beds on the north and east coasts of Singapore Island were chosen. At low tide the sand was carefully removed from the top end of each burrow so as to expose the anterior tips of the shell without loosening the lower portion of the burrow containing the rest of the animal. From the anterior end of the still attached specimen a triangular piece was removed with a nail clipper. The detached triangular piece, whose sides measured about two to three mm. each, contained both shell and mantle of both the dorsal and the ventral shell valves. After this operation the animals were covered with sand. On the following day most of the operated animals had re-formed the top part of their burrows. After a period of growth the animals were either re-notched for a further period of growth on the beach or removed to the laboratory for measurements. Experiments on large specimens were carried out on the north coast, where these were more abundant, and those on small specimens, on the east coast.

The length and width of the shell valves of fresh, wet specimens were measured with a vernier caliper to the nearest 0·01 mm. for individuals not exceeding 7 mm. in shell length, and to the nearest 0·1 mm. for all others. In accordance with usual practice (Kawaguti, 1943 ; Deleers & Pastiels, 1952), the length (L) of the shell is the chord along the sagittal plane (Fig. 1). The longest chord at right angles to L is the width (W). The width thus measured

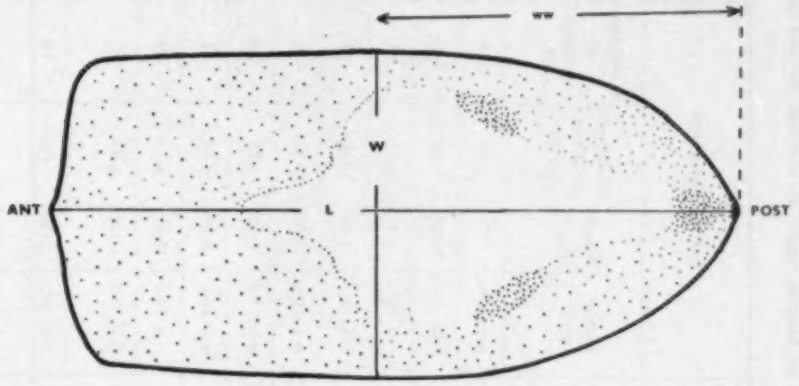

Fig. 1—The interior of the dorsal shell valve in *Lingula unguis* to show the linear measurements of length (L) and width (W). Linear measurement of the region of maximum width = ww.

appears to be a more satisfactory index than the widths taken at the thirds (Kawaguti, 1943) or the sixths (Deleers & Pastiels, 1952) of the length, since the relative position of the region of maximum width shifts with growth. The change in the relative position of the region of maximum width is studied by taking a linear measurement (ww) of this along the sagittal plane from the posterior end (Fig. 1).

RESULTS

Growth experiments on the east coast

Large specimens. Specimens notched on 31st March, 1952, were collected after seven and twelve months' growth in their original burrows (Pl. 1, figs. A & B). The length increments of the shell were plotted against initial length and a curve was fitted to the coordinates of individuals which had had an equal period of growth. These curves (Fig. 2) show that length increment is inversely proportional to initial shell length, and that the increment of the dorsal valve is less than that of the corresponding ventral valve.

Small specimens. The results of growth experiments on small specimens are summarized in Table 1. These specimens showed considerable growth in a month in spite of the disturbance caused by the removal of part of the shell.

Growth experiments on the north coast

The results of similar experiments on large specimens notched on 29th March, 1952 and collected after three, six-and-a-half and twelve months'

TABLE 1—Initial shell length and width and their increments in notched specimens of *Lingula unguis* on the east (E) and north (N) coasts of Singapore Island.

	Date of notching	Growth period (Dy = days, Yr = years)	Coast and sample size	Shell valve	Initial dimension (mm.)		Growth increment				Difference in mean increment/ standard deviation of the difference
					Range	Mean	Range mm.	Mean (mm.)	Standard deviation (mm.)	Coefficient of variation %	
Shell length	2nd July, 1952	30 Dy	E 6	dorsal	4·4– 7·0	5·8	0·9– 1·6	1·25	1·28	124	0·11
				ventral	4·6– 7·0	6·0	0·9– 1·6	1·33	1·25	115	
	22nd Oct., 1952	38 Dy	E 21	dorsal	6·1–15·9	10·8	0·9– 3·4	2·32	0·65	28	0·23
				ventral	6·6–17·5	11·1	1·4– 3·3	2·50	0·53	21	
	29th March, 1952	1 Yr	N 85	dorsal	16·4–45·5	34·4	0·4–16·2	4·35	3·78	87	0·30
				ventral	16·2–46·0	35·1	0·8–17·1	4·53	4·02	89	
	31st March, 1954	3 Yr	N 170	dorsal	19·3–44·9	35·1	1·3–19·6	6·26	4·52	72	0·60
				ventral	20·3–46·1	35·7	1·2–19·7	6·55	4·58	70	
Shell width	29th March, 1952	1 Yr	N 74	dorsal	7·4–20·0	15·5	0·1– 6·7	1·75	1·53	87	
	31st March, 1954	3 Yr	N 155	dorsal	8·7–19·6	15·4	0·6– 8·1	2·69	2·00	74	

growth in their original burrows (Pl. 1, figs. C & D ; Fig. 2) confirm the conclusions obtained by the experiments on the east coast. The increments were greater, however.

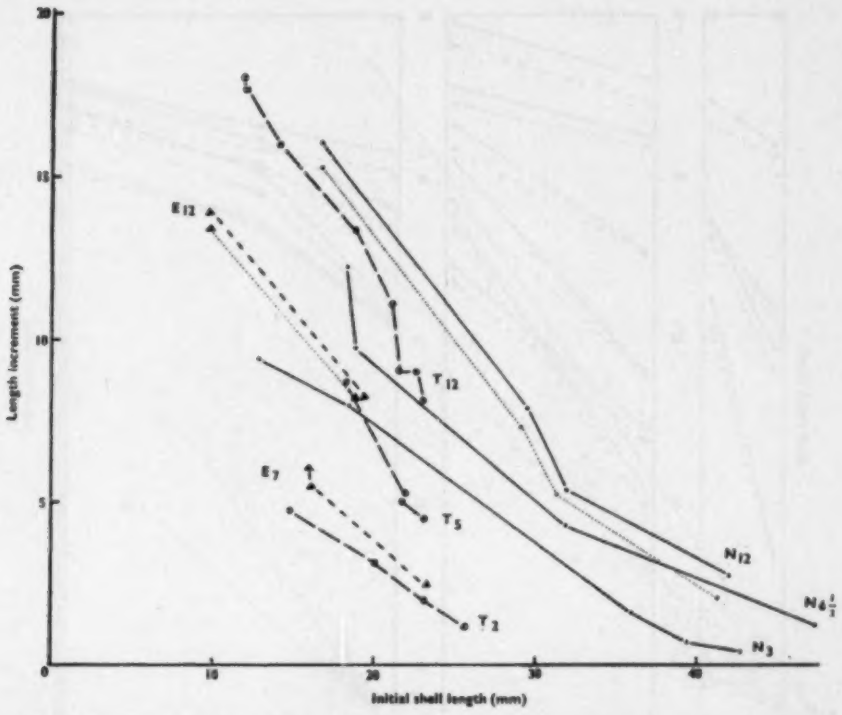

Fig. 2—Length increment and initial length of the ventral shell valves of *Lingula unguis* after 7 and 12 months' growth on the east coast (▲=coordinates of one valve, E_7 and E_{12}=after 7 and 12 months' growth on the east coast) ; after 3, 6½ and 12 months' growth on the north coast (●, and N_3, $N_{6\frac{1}{2}}$ and N_{12}) ; and after 2, 5 and 12 months of transfer to the north coast (○, T_2, T_5 and T_{12}). Dotted lines=increments of corresponding dorsal shell valves after 12 months' growth on the east and north coasts respectively.

Experiments on growth of specimens transplanted from the east to the north coast

About 500 small specimens, collected from the east coast at the level of low-water neap tide and notched on 31st March, 1952, were transplanted on the following day to the north coast at about the same intertidal level by insertion to a depth of 3 cms. below the surface in the upright position in a marked plot previously cleared of all the indigenous population. Only four specimens remained on a quarter of the experimental plot two months later, four on another quarter five months later, and eleven on the rest of the plot twelve months later. The results show that in spite of the disturbance resulting from the transfer, the transplanted notched specimens made greater length increments at corresponding initial length than the notched specimens left

to grow in their original burrows on the east coast (Pl. 1, figs. E & F). The increment curves (Fig. 2) lie between those of the east- and north-coast experiments.

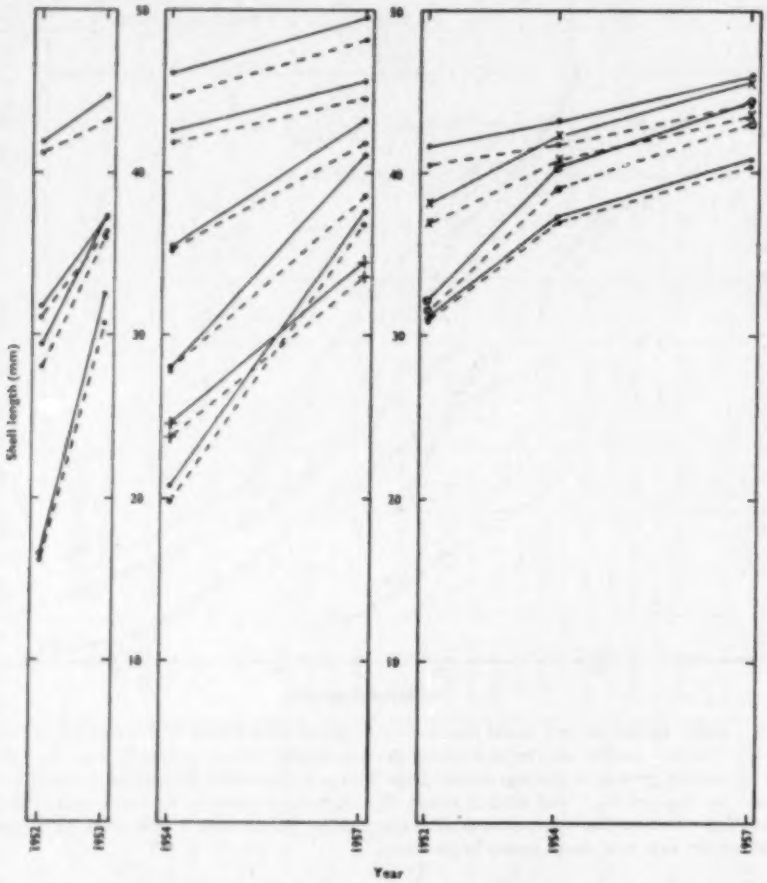

Fig. 3—Initial and final linear shell lengths of some dorsal (discontinuous line) and the corresponding ventral shell valves (continuous line) of *Lingula unguis* after 1, 3 and 5 years' growth on the north coast.

Experiments on growth over longer periods on the north coast

Large specimens notched on 29th March, 1952 were collected on 27th March, 1953 after approximately one year's growth and those notched on 31st March, 1954 were collected on 20th March, 1957 after approximately three years' growth. Another batch of specimens, notched on 1st April, 1952 and notched again on 1st April, 1954, were collected on 20th March, 1957 after almost five years' growth altogether (Pl. 1, figs. G. & H). The results show that growth

occurs in all the specimens notched and that larger and presumably older individuals have flatter curves of growth than the smaller ones (Figs. 3 & 4). Again, the length increments of the ventral valves exceed those of the corresponding dorsal valves.

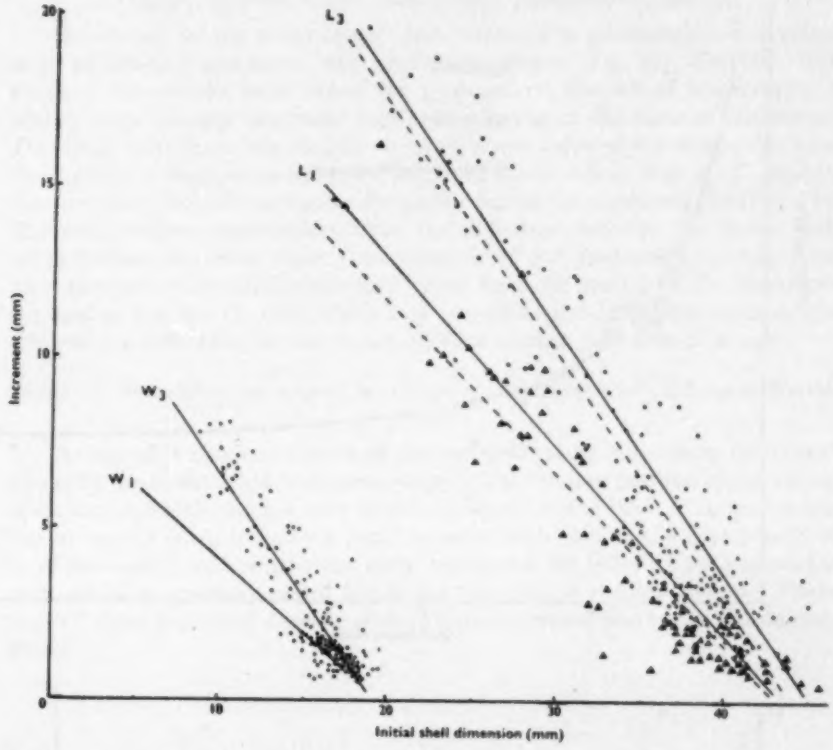

Fig. 4—Regression of length increment on initial length after 1 year's (L_1) and 3 years' growth (L_3) of 85 and 170 postlarvae respectively, and the regression of width increment on initial width after 1 and 3 years' growth (W_1 and W_3) of 74 and 155 postlarvae respectively. Ventral valves = continuous line; dorsal valves = discontinuous line. ● = coordinates for ventral valve length or width after 3 years' growth; ▲ = coordinates for ventral valve length after 1 year's growth.

The scatter diagram (Fig. 4) of increments plotted against initial lengths or widths show a negative correlation. The correlation coefficients for one year's growth in length of eighty-five specimens notched on 29th March, 1952 (Table 1) are −0·947 and −0·940 for the dorsal and ventral shell valves respectively, the corresponding values of r for three years' growth of 170 specimens notched on 31st March, 1954 (Table 1) being −0·927 and −0·928. The values of r for width increments and initial widths of the dorsal valves are −0·893 for seventy-four specimens and −0·898 for 155 specimens from the above samples after one and three years' growth respectively. After three

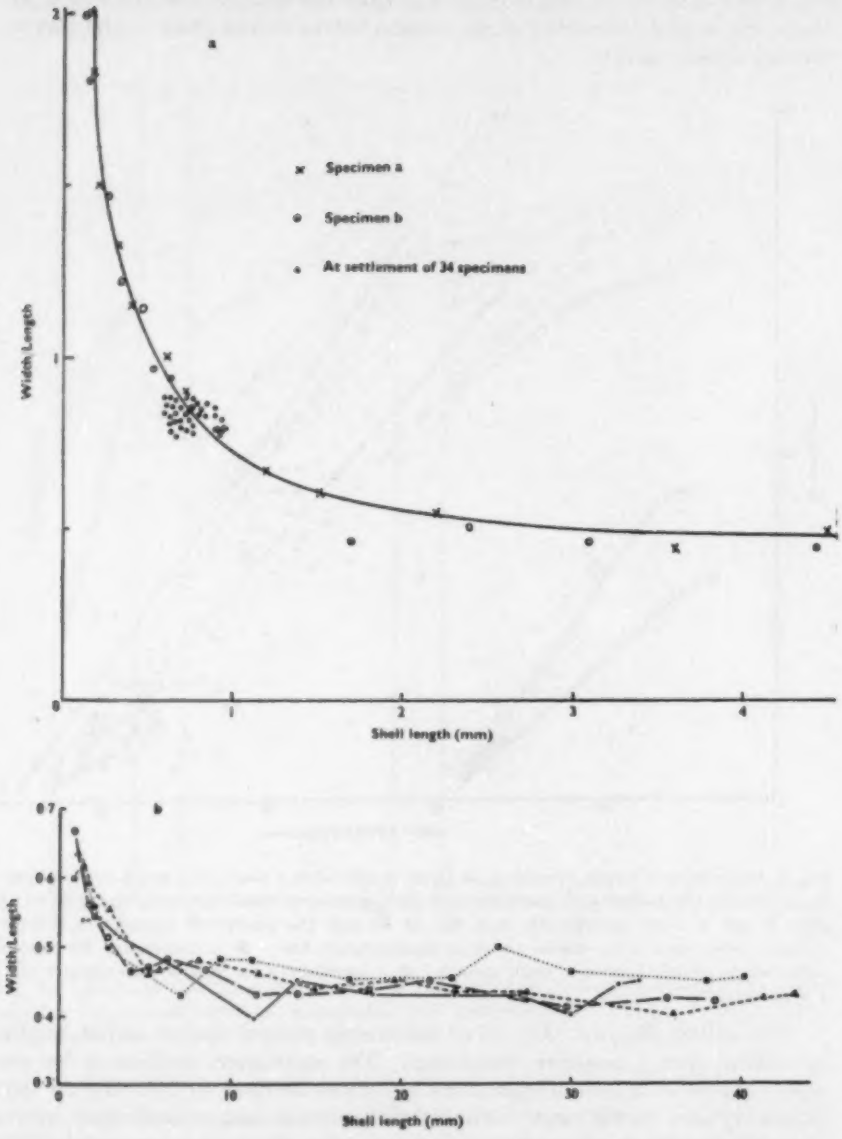

Fig. 5—Width/length ratio and dorsal valve length in *Lingula unguis*. a, Curve of drop of W/L ratio during the larval and early postlarval stage, and a scatter of W/L ratios of the larvae at the time of settlement. b, W/L ratios during the postlarval stage of four different specimens, the coordinates for which are represented by ▲, ○, ●, ■ respectively.

years' growth the regressions of length increment on initial length and of width increment on initial width are nearly parallel (Fig. 4), indicating a similar rate of decrease of increment with increasing length and width. Similarly, the regressions for one year's growth are nearly parallel (Fig. 4).

Change in width/length ratio during postembryonic growth

The change in the width/length ratio during the postembryonic development of the shell appears to be a continuous process (Fig. 5). The high W/L ratios of 1·5–2·0 characteristic of the protegula at the end of the embryonic period drops rapidly to around 0·8 for the larvae at the time of settlement. This ratio then drops less rapidly to reach a low value of 0·4–0·5 by the time the postlarval shell valve reaches a length of about 4 mm. (Fig. 5 a). Around this low value the ratio fluctuates during the rest of the postlarval life (Fig. 5 b). The ratio varies considerably, since the difference between the mean W/L ratios before and after three years' growth of 155 postlarvae ranging from 19·3–44·6 mm. in initial dorsal valve length from the sample of 170 specimens notched on 31st March, 1954 (Table 1) is 1·95 times and 2·86 times the standard error of the difference for the dorsal and the ventral valves respectively.

Change in the relative position of the region of maximum width during postlarval growth

The region of maximum width of the two shell valves in the same individual generally lies in the same transverse plane. The relative position of the region of maximum width changes with increasing length of the shell. Curves tracing this change (Fig. 6) indicate a rapid anterior shift during the first 3 mm. or so of the shell length during the early postlarval life followed by a posterior shift, which is generally halted before the total length reaches 25 mm. From then on there is a slight anterior shift, which was noted previously (Kawaguti, 1943).

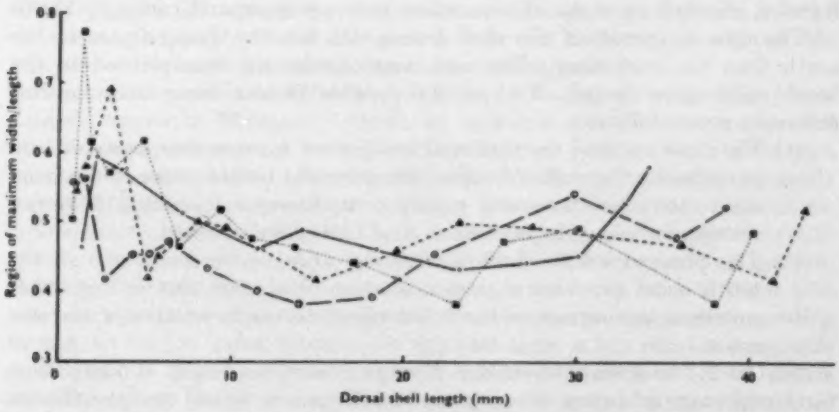

Fig. 6—Ratio of linear measurement of the region of maximum width (= ww of Fig. 1)/total shell length plotted against total shell length for the dorsal shell valves of four specimens of *Lingula unguis*, the coordinates for which are represented by the symbols ▲, O, ●, ■ respectively.

DISCUSSION

The results of growth experiments indicate that off Singapore Island the postlarval shell of *Lingula unguis* grows continuously throughout life. The length increments vary between specimens of the same initial length, as width increments do between specimens of the same initial width. Length increments also vary between the two valves of the same individual, but the differences between the mean length increments of the two opposing valves are not statistically significant, the difference between the means being in each case less than the standard error of the difference (Table 1).

The continuous growth of the shell results in the absence of any recognizable annual growth rings. However, several disturbance rings may occur in a year on that part of the shell formed during the first few years of life (Pl. 1, figs. C–H), while they are less noticeable during the last few years of life. Presumably the growth-retarding factor or factors are more effective when growth is rapid during the first few years of life, so that a slight retardation or interruption of growth, which could cause a disturbance ring in a young specimen, had no noticeable effect on a slow-growing older one. One of the possible growth-retarding factors is a drying up of the shell-forming edges of the mantle whenever low spring tides coincide with a sunny spell, since the burrows are then dried up in the sun and some of the animals have been observed to remain near the top of the burrow with the anterior end of the shell gaping.

It was not possible to distinguish the different age-groups in a large sample of *Lingula unguis* because of (1) continuous growth throughout the year, (2) continuous breeding and larval settlement throughout the year (Chuang, 1959), and (3) the merging of the animals of different age-groups during the period of slow growth when the shell length exceeds 35 mm. or so. Even in Japan, where *L. unguis* breeds from mid-July to the end of August off Misaki, Honshu (Yatsu, 1902 ; Kume, 1956), the frequency curves for body weight, shell length, width and thickness of a sample of 375 specimens from Tomioka, Kyushu, showed no signs of separation into age-groups (Kawaguti, 1943).

The rate of growth of the shell varies with locality, being faster in the north than the east coast, The east coast specimens, transplanted to the north coast, grew faster. Two of the possible factors responsible for this difference are as follows :—

(1) The clear water of the sheltered north coast favours acceptance of food particles by these filter-feeders, the generally turbid water of the more open east coast favouring rejection mechanisms (Chuang, 1956) and thereby reducing the number of food particles swallowed.

(2) The presence of the buried decaying roots of the mangrove in the north coast provides a greater amount of organic matter and other nutrients in contrast to the relatively clean sandy beaches of the east coast.

Yatsu (1902) found that the shell of *L. unguis* reached a length of 5 mm. from the larval stage in Japan in one year. On Singapore Island the growth rate is higher, an average increase of 2·3 mm. in shell length was attained by specimens of 6·1–15·9 mm. dorsal shell length in thirty-eight days on the east coast. The growth rate can be expected to be even higher on the north coast.

Presumably the different rates of growth and the environmental differences between the two localities in Singapore contribute to the following differences between the two populations of *Lingula unguis* :—

(1) The size of the female at maturity differs. On the north coast the smallest female found spawning had a ventral valve length of 22·6 mm. (Chuang, 1959), while on the east coast, 14·3 mm. Judging from the composite growth curve of Fig. 3, one presumes that the females from the north coast begin to spawn when they are about one to one-and-a-half years old.

(2) The maximum shell length varies. On the north coast many specimens exceed 50 mm. in ventral valve length, the longest specimen found since 1952 being 52·2 mm. On the east coast the longest specimen found had a ventral valve length of 44·3 mm., the maximum length having dropped in recent years to around 35 mm.

(3) Specimens of equal shell lengths differ in age. Due to the slower growth on the east coast, a certain shell length is reached much later here than on the north coast. Hence a shell of certain length is younger in age on the north than the east coast.

(4) Longevity differs.

The longevity of *Lingula unguis* has been a matter for conjecture. François (1891) found that *L. unguis* in Nouméa varied in length between 15–50 mm. and believed that it lived more than one year at the least. Morse (1902) distinguished a certain proportion of thickened, discoloured and eroded shells from other equally large but thinner, greener and more perfect ones collected at the same time in Japan. Inferring that the thickened shells did not belong to the same year's brood, he concluded that the life span was two or more years. Yatsu (1902) found that it took one year for the larvae to reach a shell length of 5 mm. Estimating that *L. unguis* would take seven years to reach a maximum length of 35 mm. at Misaki at the same growth rate, he concluded that the average life span was five years or more. Although this conclusion is based on a wrong assumption that the growth rate was the same throughout life, it is nearer the truth than the estimations of other authors.

It was not possible to follow the shell growth during the entire life span of *Lingula unguis* in Singapore. However, sufficient specimens were observed over a period of five years in their original burrows with only two notchings, which obviously exceeded in severity the effect of whatever caused the normally occurring disturbance rings in some shells. The results of these observations, figured side by side with others in Fig. 3, seem to represent a probable composite growth curve of *L. unguis* on a beach fairly favourable for maximum growth. Based on this composite picture the life span on the north coast is about nine years for specimens of 45 mm. and about twelve years for specimens exceeding 50 mm. in ventral valve length. On the east coast a less reliable composite growth curve, based on growth experiments over periods of six to twelve months, appears flatter and seems to indicate a life span of six to eight years for a specimen of 35 mm. dorsal valve length.

The transformation from the transversely oval to the longitudinally oval shape of the shell valve during the larval and early postlarval stages is reflected

in a rapid change of the W/L ratio, which is halted at the stage of 4 mm. postlarval shell length. Although the ratio fluctuates around a low value of 0·4–0·5 in the larger postlarvae, it varies so much that the difference between the mean ratio of the same 155 large postlarvae before and after three years' growth is statistically significant. The difference between the mean ratios of two independent samples can be expected to be even greater. Johnston & Hirschfeld (1920) found that the ratio was fairly constant and could be used for the separation of recent *Lingula* species that closely resemble one another, but the results given in this paper show that this is not true.

The change in shape from the circular larval shell to the longitudinally oval postlarval shell is accompanied by a rapid anterior shift in the relative position of the region of maximum width in the young postlarva. Another anterior shift occurs in late postlarval life, thus confirming Kawaguti's observation (1943).

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EXPLANATION OF PLATE

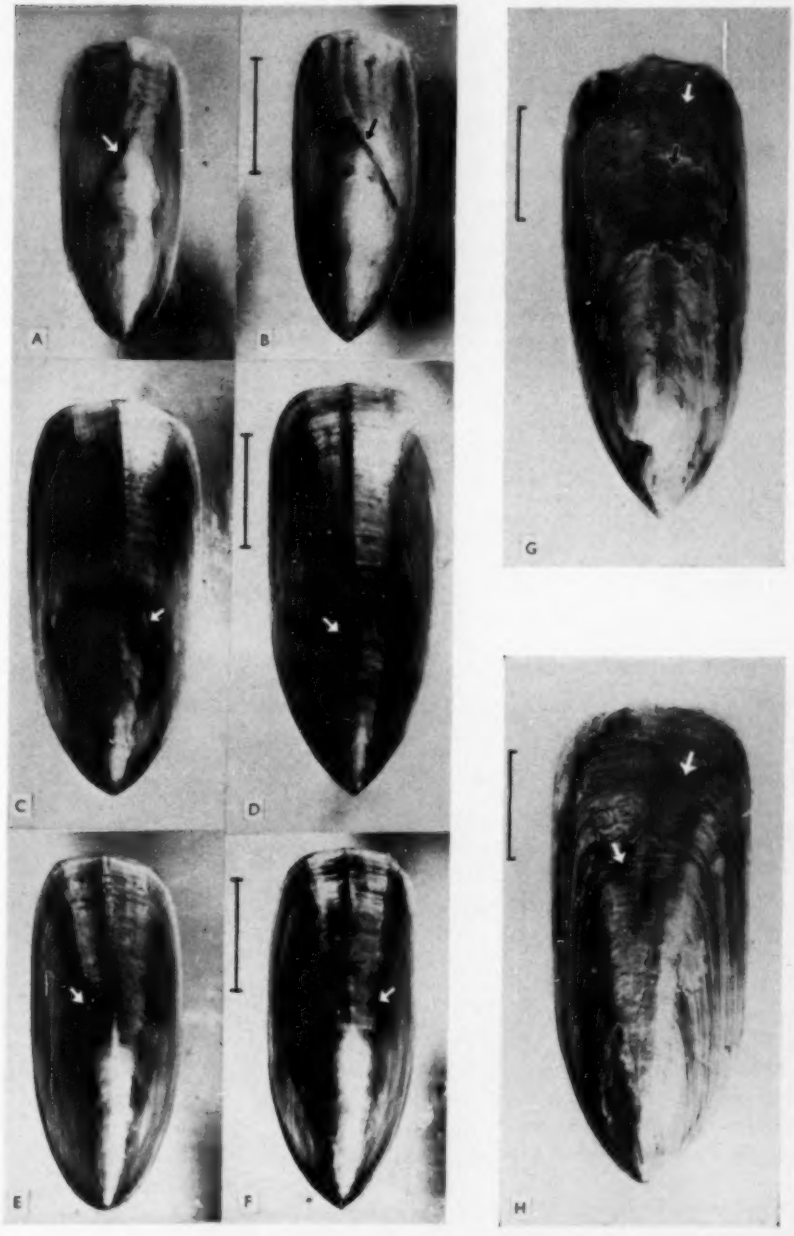
Scale for all figures = 10 mm. The arrow indicates where a piece of the shell has been removed.

Figs. A and B. Dorsal and ventral shell valves of the same specimen of *Lingula unguis* after one year's growth on the east coast.

„ C and D. Dorsal and ventral shell valves of another specimen after one year's growth on the north coast.

„ E and F. Dorsal and ventral shell valves of another specimen, transplanted from the east coast, after one year's growth on the north coast.

„ G and H. The ventral valve and the dorsal valve of two different specimens notched in 1952 and again in 1954, and collected in 1957, after five years' growth on the north coast altogether.



Growth of the postlarval shell in *Lingula unguis* (L.).

OBSERVATIONS ON THE LAYING AND HATCHING OF OCTOPUS EGGS IN THE SOCIETY'S AQUARIUM

BY

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(Accepted 13th June, 1961)

Three different individuals of *Octopus vulgaris* laid eggs in the Society's Aquarium in September 1957, May 1960 and August 1960 respectively. Mating was observed in the first instance. The eggs were brooded for a period of thirty-nine to forty-two days. The larvae hatched successfully, but did not feed, although offered a variety of foods, and survived for an average of eight days. In each case the mother died immediately or shortly after the hatching of the last larvae. The behaviour of the female during brooding is described.

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INTRODUCTION

There have been few observations on the breeding behaviour and egg-laying of octopods, and even fewer cases of octopus eggs hatching in captivity. Aristotle wrote an account of the breeding of octopus in the Mediterranean, the details of which have often been held in doubt, although recent work has repeatedly confirmed many of his statements. The mating behaviour of *Octopus vugaris* has been described by Racovitza (1894), and the egg-laying by Heldt (1948). One of the most noteworthy accounts is that of Lee (1875), who observed the mating, laying and hatching of eggs of *O. vulgaris* in the Brighton Aquarium in 1873 and 1875. Naef (1928) records the laying and hatching of *O. vulgaris* eggs in the aquarium of the Stazione Zoologica at Naples in 1911–13, and eggs were laid in the Plymouth Aquarium in 1947 (Rees, 1950, plate I). Two species of octopus from the coasts of California have been seen to lay eggs in captivity, namely *O. apollyon* (Fisher 1923) and *O. bimaculatus* (Fox 1938). In the Southern hemisphere Le Souef & Allen (1933 & 1937) have recorded observations on *O. cyaneus* and on another species of octopus, in one case with successful hatchings, and Batham (1957) has reported the laying and hatching of the eggs of *O. maorum* in an aquarium tank at the Portobello Marine Biological Station.

The lesser octopus, *Eledone cirrhosa* has mated and laid eggs in the aquarium at Banyuls-sur-Mer (Joubin 1888), and eggs have also been laid in the aquarium

at the Port Erin Marine Biological Station (Gravely 1908), but in neither case did they hatch.

OBSERVATIONS IN 1957

A collection of marine animals from Madeira arrived at the Society's Aquarium on 12th July 1957, and included two specimens of *Octopus vulgaris*. These were placed on exhibition in a tank which is reserved for invertebrates, and which is supplied with fresh sea water which has not been in contact with the water circulating throughout the remainder of the Sea Water Hall. One of the new octopus was considerably larger than the other and could also be readily distinguished by the possession of short healed stumps in the place of two of the eight tentacles.

Courtship and egg-laying

In the period between mid-July and the end of August some courtship activity, as described by Racovitza (1894), was noticed and mating was observed on 20th to 21st July. Each octopus took up its own permanent position beneath an overhanging ledge ; the largest of these shelters was firmly held by the larger octopus, here distinguished as A. On 6th September this specimen started to lay eggs, and continued to do so at intervals during the next four days. The eggs were laid in batches of several strands at a time and attached to the underside of the overhanging ledge. About fifty to seventy strands of eggs were laid.

On 11th September the second octopus climbed out of the tank. Autopsy showed that it was a spent male.

Brooding of the eggs

After the eggs had been laid the mother positioned herself with the main part of her body lying immediately below the eggs. She attached herself to the overhanging ledge by bending the arms over the body in such a way that the suckers were facing upwards and were able to grip the under surface of the ledge. The mouth was kept facing outwards and at least one eye was visible at any one time.

The mother remained in this position throughout the period of brooding, only leaving on rare occasions to remove an intruding starfish or sea-urchin. During this period she squirted water from her siphon over the eggs at varying intervals. Somethimes there were bursts of water-squirting followed by periods of rest. At such times the fine tips of two or three tentacles were run through the egg clusters. This activity has been recorded by other observers.

A few strands of eggs were removed from the main mass the day after the laying ceased. These strands were kept in a tank with well-aerated water but showed no signs of development during the next few days.

The rate of siphon discharge increased considerably during brooding. Other octopus kept in the same conditions have an average rate of twenty-nine siphon beats per minute, with a range of twenty-seven to thirty-two. During

most of the brooding period the average rate of siphon beat of the mother octopus was forty per minute, with a range of thirty-eight to forty-three.

Several attempts were made to feed the brooding octopus with shore crabs (*Carcinus moenas*). On nearly every occasion the crab was picked up with the tip of a tentacle, and deposited in an area distant from the eggs. Occasionally a single crab must have been eaten during the night as an empty shell would be found the following morning. This agrees with the suggestion of Batham (1957) who writes " In general, it seems that the normal direct food response to a live crab is inhibited, but that total starvation during brooding does not necessarily occur ".

Hatching of the eggs

The eggs started to hatch on 16th October and continued to do so in batches over the next twelve days, giving a brooding period of forty-one to fifty-two days.

Immediately after hatching the young octopus swam upwards in the tank and towards the light. After three to four hours they sank and remained in a mass about 20 cm from the bottom of the tank. Attempts were made to feed them on harpacticid copepods, *Artemia*, ciliates and yeast cells but no feeding was observed either in small aquaria or when viewed under the binocular microscope ; the natural food of the larvae is unknown. The majority of the larvae lived, presumably on the yolk in their guts, for eight to nine days. This is comparable with the survival of newly-hatched octopus larvae at the Naples Aquarium (Naef, 1928).

Death of the mother

After the last eggs had hatched the mother left her position under the overhanging ledge and moved slowly about the tank. She no longer fed, even at night, and her body and tentacles appeared extremely emaciated. Finally she died on the morning of 4th November.

OBSERVATIONS IN 1960

A single octopus (here distinguished as B), arrived in the aquarium on 15th April 1960, and was immediately placed on exhibition, without any companion, in the tank originally occupied by octopus, A. There was thus no chance of mating taking place in captivity.

On 26th May this octopus started to lay eggs in strands attached to the underside of the same overhanging ledge as was used by octopus A in 1957. Egg-laying was completed by 29th May. The behaviour of the mother during the brooding period was exactly the same as that of octopus A. Her eggs started to hatch on 3rd July and continued to do so until the morning of 9th July, when she died.

A further female (C) which arrived from Madeira on 10th June 1960, laid eggs on 12th August and during the following four days. These eggs hatched between 23rd and 29th September and the female died on 3rd October. Table 1 gives comparative data for the breeding in 1957 and the two occasions in 1960.

Table 1—Summary of events.

	Octopus A 1957	Octopus B 1960	Octopus C 1960
Arrival	12th July	15th April	10th June
Courtship and mating	20–21st July	—	—
Egg-laying started	6th September	26th May	12th August
Hatching started	16th October	3rd July	23rd September
Hatching finished	28th October	9th July	29th September
Death of mother	4th November	9th July	3rd October
Brooding period (in days) (1st lay to 1st hatch)	41	39	42
Frequency (per minute) of siphon discharges	40	38	41
Mean temperature of water in tank.	23°C	22°C	22°C

DISCUSSION

Courtship and mating were only observed in one octopus (A) when the period between mating and the laying of the first eggs was forty-seven days. The other two octopus must have mated before arrival in London. The period of brooding was remarkably consistent (41, 39 and 42 days).

The failure of the larvae to feed, despite the offer of a variety of foods, agrees with the experience of Naef (1928) at Naples. It appears that the larvae of *O. vulgaris* have never survived under aquarium conditions for more than eight to twelve days.

In the sea it is known that the larvae are pelagic for a considerable time. The observed sinking of the larvae a few hours after hatching should probably be regarded as a reaction to the amount of illumination and not as a movement towards the bottom.

The death of the mother in each case after the hatching of the last eggs would suggest that this may happen also in nature. Certainly the death could not be ascribed to any change in the conditions in the tank and several non-breeding males and female octopus have been kept in the same tank for periods up to six and occasionally even eight months.

ACKNOWLEDGMENT

I am much indebted to Mr B. B. Boycott for reading and criticising the manuscript of this paper.

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NOTES AND ABSTRACTS

MEETINGS OF THE SOCIETY FOR SCIENTIFIC BUSINESS

14th February 1961

Dr F. C. Fraser, F.L.S., in the Chair

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE: DECEMBER 1960 AND JANUARY 1961

By L. Harrison Matthews, M.A., Sc.D., F.R.S.

The registered additions (mammals and birds) to The Zoological Society's collection in London during the months of December 1960 and January 1961 were 136 in number. The following are of special interest: the collection of animals collected in Madagascar by Mr David Attenborough and presented by the Television Service of the British Broadcasting Corporation which included a ruffed lemur *Lemur variegatus*, ten mouse lemurs *Microcebus murinus*, a tailless tenrec *Tenrec ecaudatus*, and seven spiny tenrecs (two *Setifer s. setosus* and five *Setifer setosus nigrescens*, a dwarf mannikin *Spermestes nana*, nine Madagascar weaver birds *Foudia madagascariensis*, a cinnamon roller *Eurystomus g. glaucurus*, a vasa parrot *Coracopsis vasa*, and five Madagascar lovebirds *Agapornis cana*. The cinnamon roller is new to the collection; a pair of pygmy marmosets *Cebuella pygmaea*, and a male talapoin monkey *Miopithecus talapoin*, purchased, and a golden cat *Felis temmincki* presented by Mr E. P. Gee, C.M.Z.S.; two pumas *Felis concolor* and two Syrian bears *Ursus arctos syriacus*, born in the menagerie; a Christmas Island frigate bird *Fregata andrewsi*, presented by Captain D. Scorgie; a yellow-plumed honey-eater *Meliphaga ornata*, a specimen of which has not been in the collection for many years, was received in exchange, and an Indian blue robin or chat *Luscinia brunnea*, new to the collection, presented by Messrs G. H. and J. R. Newmark.

The following communications were read and discussed:

Professor A. J. E. Cave. The retrolingual gland of the elephant. [Published in the *Proceedings*].

Dr W. C. Osman Hill. Observations on the Platyrrhine genus *Brachyteles*.

Dr Desmond J. Morris and Mr Jan Van Hooff. The concept of submission.

Mr W. H. Potts. Film of game animals of E. Africa, with remarks on their role as hosts of the tsetse fly. [Film] by Dr P. Glover, Tsetse Control Dept., Veterinary Services, Kenya].

14th March 1961

Dr Errol I. White, C.B.E., F.R.S., in the Chair

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE : FEBRUARY 1961

By L. Harrison Matthews, M.A., Sc.D., F.R.S.

The registered additions to the Society's collections of mammals, birds, reptiles and amphibians during the month of February were 63 in number. The following may be specially mentioned : a brown stump-tailed monkey *Macaca speciosa* and a ruddy mongoose *Herpestes smithi*, both of which have not been represented in the collection for several years, presented by Dr K. C. Searle ; fourteen blackbuck *Antilope cervicapra* purchased; a female American bison *Bison bison* and a Bennett's Wallaby *Protemnodon rufogrisea frutica*, born in the Menagerie ; four spiny tenrecs *Setifer setosus nigrescens* also born in the Menagerie, the first time this species have bred here ; two Hawaiian geese *Branta sandvicensis* presented by The Wildfowl Trust ; a diamond python *Python spilotus*, purchased ; three Sganzin's tree boas *Corallus madagascariensis*, born in the Menagerie ; three Malayan giant toads *Bufo asper*, presented by Dr W. S. Bristowe. During the same period there were 60 additions to the Collection at Whipsnade Park, including some domestic animals and excluding transfers from London. Of particular interest were : the two Polar bears *Thalarctos maritimus*, six brown bears *Ursus arctos* and three bactrian camels *Camelus bactrianus*, all born in the Park.

The following communications were read and discussed :

Dr K. M. Backhouse and Dr P. J. G. Smart. The mechanism of wave riding in porpoises. [Published in the *Proceedings*].

Dr B. Morris. Some observations on the breeding season of the hedgehog and the rearing and handling of the young. [Published in the *Proceedings*].

Professor Ruth E. M. Bowden and Dr J. L. Scheuer. Comparative studies of the nerve supply of the larynx in eutherian mammals. [Published in the *Proceedings*].

Dr Fae Hall. Locomotion in vertebrates. [Illustrated by a film].

11th April 1961

Dr Errol I. White, C.B.E., F.R.S., in the Chair

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE : MARCH 1961

By L. Harrison Matthews, M.A., Sc.D., F.R.S.

The registered additions to the Society's collections of mammals, birds, reptiles and amphibians during the month of March were 63 in number. The

following may be specially mentioned : a young serval *Felis serval*, presented by Mr J. R. Story ; a female mandrill *Mandrillus sphinx*, purchased to make up a suitable pair of this species ; a female orang-utan *Pongo pymaeus* born in the menagerie, the first time the birth of an orang-utan has been recorded here ; two ring-tailed lemurs *Lemur catta* and a Malaysian fruit-bat *Cynopterus brachyotis* also born ; two male Kori bustards *Choriotis kori struthiunculus*, presented by Mr J. O. D'eath, F.Z.S. During the same period there were 25 additions to the collection at Whipsnade Park. The following are of special interest : a collared peccary *Tavassu tajacu* and a Thomson's gazelle *Gazella thomsoni*, born in the Park.

The following communications were read and discussed :

Mr R. W. Hayman. The Red goral of the North-east Frontier Region. [Published in the *Proceedings*].

Mr E. R. A. Turner. Survival values of different methods of camouflage as shown in a model population. [Published in the *Proceedings*].

Professor Thomas W. M. Cameron. The phylogeny of Southern Hemisphere mammals based on their parasites.

9th May 1961

Professor E. C. Amoroso, M.B., B.Ch., Ph.D., F.R.S., in the Chair

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REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE : APRIL 1961

By L. Harrison Matthews, M.A., Sc.D., F.R.S.

The registered additions to the Society's collections of mammals, birds, reptiles and amphibians during the month of April were 96 in number. The following may be specially mentioned : a male cheetah *Acinonyx jubatus*, a magnificent specimen, presented by Mr and Mrs N. de B. Priestley and Mr and Mrs G. Strong of Nigeria ; three great eagle owls *Bubo bubo* and two spotted eagle owls *Bufo africanus* hatched in the Menagerie and an Imperial parrot *Amazona imperialis* deposited ; a collection of reptiles and amphibians from North America which included a pygmy rattlesnake *Sistrurus miliarius* and two narrow-mouthed toads *Gastrophryne carolinensis* presented by Messrs G. H. and J. R. Newmark. During the same period there were 40 additions to the collection at Whipsnade Park. Of special interest are : two collared peccaries *Tavassu tajacu*, a white-tailed gnu *Connochaetes gnou*, a yak *Bos grunniens*, a Cotton's reticulated giraffe *Giraffa camelopardis cottoni* × *G. c. reticulata*, a hippopotamus *Hippopotamus amphibius*, all born in the Park ; and six red pandas *Ailurus fulgens*, received in exchange.

The following communications were read and discussed :

Mr O. Graham-Jones and Dr W. C. Osman Hill. Pregnancy and parturition in an orang-utan in the Society's Gardens, with observations on the placenta.

DR E. T. B. FRANCIS. The sources and nature of salivary secretions in Amphibia. [Published in the *Proceedings*].

PROFESSOR J. M. DODD and DR C. K. GODDARD. Some effects of oestradiol benzoate on the reproductive ducts of female dogfish (*Scyliorhinus caniculus*). [To be published in the *Proceedings*].

13th June 1961

SIR LANDSBOROUGH THOMSON, C.B., O.B.E., M.A., D.Sc.,
Vice-President in the Chair

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE: MAY 1961

By L. HARRISON MATTHEWS, M.A., Sc.D., F.R.S.

The registered additions to the Society's collections of mammals, birds, reptiles and amphibians during the month of May were 118 in number. The following may be specially mentioned: eight lar gibbons *Hylobates lar*; four red-faced uakari monkeys *Cacajao rubicundus*; a Malayan pangolin *Manis javanica*, new to the collection; four Malay tupaias *Tupaia glis ferruginea* and a male lion *Panthera leo* all purchased; an Indian porcupine *Hystrix indica*, all presented by Mr G. B. Eastmure; an Egyptian mongoose *Herpestes ichneumon*, presented by Mr R. J. Powditch; an eight month old male lion cub *Panthera leo*, presented by Arnhem Zoo; two Montezuma oropendolas *Gymnostinops montezuma*, three rufous laughing thrushes *Dryonastes poecilorhyncha berthemyi*, and one black-eared wheatear *Oenanthe hispanica hispanica*, the last three species are new to the collection, six grey amphisbaenas *Blanus cinereus*, purchased; a northern blue-tongued skink *Tiliqua scincoides*, born in the menagerie; a braided tree frog *Chiromantis xerampelina* and two spotted tree frogs *Hylambates maculatus*, presented by Dr I. Whimster. During the same periods there were 47 additions to the collection at Whipsnade Park. The following are of special interest: two pumas *Felis concolor*, three white-bearded gnu *Connochaetes taurinus albojubatus*, a Père David's deer *Elaphurus davidianus*, two moose *Alces alces*, a hog deer *Axis porcinus* and an East African eland *Taurotragus oryx pattersonianus*, all born in the Park and seven Père David's deer *Elaphurus davidianus*, received in exchange.

The following communications were read and discussed:

MR R. E. MOREAU. Taxonomic realism. [To be published in the *Proceedings*].

DR J. C. COULSON and DR E. WHITE. An analysis of the factors influencing the clutch size of the kittiwake. [Published in the *Proceedings*].

DR R. BASSINDALE. The marine fauna of Ghana. [To be published in the *Proceedings*].

DR W. C. OSMAN HILL. Hybridization in marmosets.

The series of colour transparencies exhibited were received from Mrs D. Boulter of Singapore and depict her hybrid marmoset and its parents (*Mico argentatus* ♂ ; *Hapale jacchus* ♀).

Hybridization has seldom been reported among the numerous species of Hapalidae, the only ones traced being (i) several *H. jacchus* × *H. pencillata* born in Regent's Park in the early 1950's and (ii) *Hapale jacchus* ♂ × *Mico argentatus* ♀ bred by English and exhibited at a Scientific Meeting of the Society in 1932 (*Proc. zool. Soc. Lond.* p. 1079) unfortunately without published description. Twin foetuses were found at autopsy on a female emperor marmoset (*Leontocebus imperator*) which had been caged with a moustached tamarin (*L. mystax*) (Lima, 1945).

In the present instance the male parent had been in Mrs Boulter's possession for six years, and had been mated for three years without result to a female lion marmoset (*Leontideus rosalia*). Then he was given as mate a female common marmoset (*H. jacchus*). Twins were unexpectedly born of this mating on the 9th June 1960 ; the two babies were found lying on the sand tray beneath the cage. To avoid contamination with human odour, they were transferred with a spoon whereon the male parent, as is usual in marmosets, took them over. He shook off the female twin which subsequently died, but

	Mico argentatus ♂	Hybrid ♂	*Hapale jacchus* ♀
Face	Naked, unpigmented, bright flesh colour.	Slightly hairy. Pale at first, dusky pigmentation developing later.	Sparsely clothed with short white hairs ; pigmentation present but individually variable.
Ears	As face. No circumaural corolla.	Pale and slightly furred, but no corolla.	Slight dusky pigmentation, and sparse black hairs. A prominent preauricular white corolla.
Body Pelage	Typically shining silvery white, except tail which is black.	As father, but with some dark hairs interspersed giving general light-grey appearance. Tendency to transverse banding on loins.	Dorsal hairs grey, annulated with brown and black, the black tending to become aligned in transverse bands, especially over loins.
Genitalia	Pendulous bilobate glandular scrotum dense opaque white contrasting with bright crimson surrounding integument.	Scrotum a tiny swelling (? immature).	[Juvenile and immature males *H. jacchus* show a globular scrotum but maturity involves dorsal bilobar extension on perineal side recalling glandular labia of ♀♀]. (Hill, 1957).

the male offspring was retained and passed to the mother at feeding times (again following the usual hapaline practice).

At seven months old the hybrid young was virtually fully grown and from the photographs appears to resemble its father in general appearance rather than its mother. However, it is described (*in litt.*) as more furry than either parent and has lighter irides than its father's (which are black).

Nevertheless, from the table above, it can clearly be assessed as intermediate in several details. From these it may safely be inferred that tendency to pigmentation of naked areas is dominant to the nonpigmented paternal condition, while the (presumably more primitive) absence of aural adornment is dominant to its presence.

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ENGLISH, W. L. (1932). Exhibition and remarks upon hybrid marmosets. *Proc. zool. Soc. Lond.* **1932** : 1079.

HILL, W. C. O. (1957). *Primates* **3** : 287.

LIMA, E. DA C. (1954). *Mammals of Amazonia* **1** : 221.

OBSERVATIONS ON THE NEST BUILDING BEHAVIOUR OF ARMADILLOS

By JOHN F. EISENBERG*

Department of Zoology University of California, Berkeley

A pair of nine-banded armadillos, *Dasypus novemcinctus*, was studied for six months at Berkeley in a preliminary analysis of the behaviour patterns displayed by this North American edentate. The nest building behaviour of this species was singled out for special consideration. Detailed notes and motion pictures were utilized in the analysis. In September of 1959, the author was allowed to observe specimens from five genera of the Dasypodidae in captivity at the London Zoo. There, and in Berkeley, the nest building response was elicited by merely placing a bundle of straw into the cage with the animal.

The postures and pattern of movement

In *Dasypus novemcinctus* the gathering and transport activities seem to be intensified by cold, but if straw is available the animals will readily build a nest at 27°C air temperature.

The complete behavioural sequence is as follows : (1) Walking about the cage and contacting the straw pile ; (2) Forward and backward clawing with the forepaws, gathering the straw under the body ; (3) Holding the straw pile under the body with the forepaws while rising on the hind legs ; (4) Hopping to the nest box sometimes forwards but usually backwards, while switching the tail from side to side ; (5) Dropping the bundle in the nest box or at its entrance and often kicking backwards as the animal moves away.

This sequence is performed smoothly and rapidly by an apparently strongly-motivated animal ; however, certain variations may occur at any step in the

*The author wishes to acknowledge the efforts of Dr Desmond Morris, Curator of Mammals at the London Zoo, whose kindness permitted the comparative study to be carried out on the specimens under his supervision.

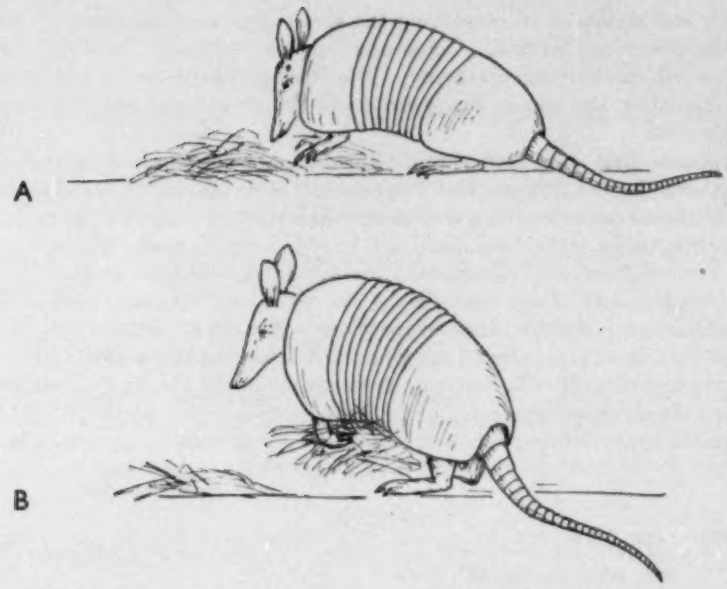

Fig. 1—A. Armadillo contacts the straw and gathers it under the body. B. Armadillo holds the straw under its body while rising on its hind legs.

sequence. These variations include : (a) gathering the straw under the animal and instead of holding the straw and hopping, turning and pushing it with the nose ; (b) after the straw has been gathered, using a tripod slide by propelling itself with the hind legs and sliding on the straw bundle ; (c) walking rather than hopping as the animal moves backwards ; (d) instead of carrying or kicking the straw into the nest box, depositing it at the entrance and pushing it in as the animal enters the box.

Variation (a) is really a break in the average sequence after Step 2. Variations (b) and (c) are breaks in the sequence after Step 3, with walking employed rather than hopping. In the case of (d) the sequence is complete except for the position where the straw is deposited—in this case outside the box. The pushing with the head may be an accidental consequence of turning and entering the nest box.

The thermoregulatory control of this animal is poor (Talmadge & Buchman). Coupled with this is the paucity of hair, resulting in a distributional limit for *Dasypus* set by the average low temperature for a geographical area. The selection for a strong behavioural mechanism to carry insulating litter into a burrow would seem to be a logical adaptation.

Nest building behaviour in other species

During the observations at the London Zoo, the air temperature and humidity were rather high. This may have depressed the overall activity of the animals ; however, *Dasypus novemcinctus* and *Tolypeutes* sp. did gather

the straw and transport it, employing the same movement sequence outlined under the preceding section. *Tolypeutes* hopped backwards only twice and did not switch its short tail markedly. The other genera observed which were not seen to gather and transport the straw included *Priodontes*, *Euphractus* and *Chaetophractus*.

It appears that two very specific patterns are coordinated in the nest building behaviour of *Dasypus* and *Tolypeutes*. First, the raking movements of the forelimbs are performed in a very stereotyped manner ; second, the hopping backwards with the straw held under the body is a rigidly performed unit even though the transport may occasionally involve a different form of locomotion. Since *Dasypus* and *Tolypeutes* belong to two very distinct subfamilies, Dasypodinae and Tolypeutinae respectively, (Simpson, 1945), one could conclude that the action patterns of gathering nesting material and transporting it are very specific self-differentiating mechanisms in the family Dasypodidae. However, the necessary ontogenetic studies to determine the relative role of the environment in the differentiation of this behaviour in both forms remain to be done.

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SIMPSON, G. G. (1945). The principles of classification and a classification of mammals. *Bull. Amer. Mus. nat. Hist.* **85** : 1–350.

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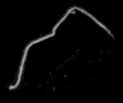
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